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Foreword

This report is the result of the first large Danish research effort on macroalgae utilization for energy purposes. The idea was developed in the beginning of 2007 during discussions between researchers at National Environmental Research Institute and Danish Technological Institute. The result of the discussions was an application for funding to the Danish government-owned company Energinet.dk which is responsible for research activities within environmental-friendly electricity production in Denmark.

The application was approved by Energinet.dk with a total budget of 10.5 million DKK and with a funding from Energinet.dk of 8.5 million DKK. The project was running from April 2008 to October 2011. The partners in the project are:

1. Aarhus University Department of Bioscience (former National Environmental Research Institute, Aarhus University)
2. Risø DTU (DTU is Technical University of Denmark)
3. DONG Energy A/S, the largest utility company in Denmark
4. Danish Technological Institute.

The contract holder is Danish Technological Institute.

The background for this research activity is that the 2020 goals for reduction of the CO_2 emissions to the atmosphere are so challenging that exorbitant amounts of biomass and other renewable sources of energy must be mobilised in order to – maybe – fulfil the ambitious 2020 goals. The macroalgae is an unexploited, not researched, not developed source of biomass and is at the same time an enormous resource by mass. It is therefore obvious to look into this vast biomass resource and by this report give some of the first suggestions of how this new and promising biomass resource can be exploited.

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1 Overview and Conclusions

1.1 Introduction

The escalating demand for energy intended for heating, electricity and transport puts a severe pressure on the world's resources of both fossil and renewable energy. Development of alternative, renewable sources of solid and liquid fuels will be vital to meet the future energy needs and help to facilitate compliance with mandated CO_2 reductions.

In Denmark, there is a long tradition of using solid biofuels such as land-based forestry and agriculture crops residual like wood and straw, and the competition for solid biomass for combustion is high. However, production of plant biomass is limited in Denmark due to unavailability of land and due to its competition with other political priorities for land-use. Massive import of wood for energy purposes is the result. World-wide, the production of bioethanol from agricultural products to the transport sector has increased significantly in USA, Brazil and to some extend in Europe (Licht 2010). The price of wheat, corn and sugar beet, that are all feedstocks used in first generation biofuel production, is influenced by the use of the products as food. Production of first generation bioethanol is based on starch feedstocks and has yet unpredictable and potential fatal consequences for the food production. In a report from 2007, United Nations emphasizes that the use of agricultural products for energy purposes leads to an increase in the market price on major biofuel feedstocks, e.g. grain, maize, rapeseed oil, soya bean, etc. which all comprise the basic diet for most of the world's population and in particular the poorest part of the population. An alternative to producing bioethanol from agricultural products is to use organic waste, straw and wood (second generation bioethanol) where the lignocelluloses go through subsequent processes of pretreatment, enzymatic hydrolysis and fermentation of C6 and C5 sugars. This second generation technology is now in demonstration scale and under development for implementation in full scale production (Larsen et al 2008).

Macroalgae comprise a vast number of photosynthetic aquatic plants and represent a huge unexploited potential for energy production. Macroalgae use light as energy source and seawater as a growth medium, capturing dissolved CO_2 and nutrients. This bioremediation capacity increases the potential value of the macroalgae biomass. In Europe, a large part of the front research in the cultivation as well as the energy conversion of macroalgae takes place in Britain thanks to their history of utilization of seaweeds. In Ireland, there has been a political will to strengthen the cooperation between research and industry in order to increase employment, export and wealth from seaweed. The Irish Seaweed Research Group at the National University of Ireland, Galway (NUIG) that was founded in 1994, has in this respect gathered large experience in off-shore cultivation of large brown algae on long lines. The group has developed a brown algae hatchery and has over the last years tested and developed the off-shore on-growth of brown algae (Edwards and Watson, 2011), also as part of Integrated MultiTrophic Aquaculture (IMTA), and participates in a large number of European projects on energy from macroalgae (http://www.irishseaweed.com/).

By aquatic biomass production, the production per hectare of biomass can be increased dramatically. Of the macroalgae studied so far, *Ulva lactuca* has the highest annual yield (up to 45 tons dry matter (DM) per hectare is documented in this project) (Nikolaisen et al 2010), a high assimilation of CO_2 as well as a high content of carbohydrates (up to 60% of dry matter). Macroalgae have characteristics that are equivalent to agricultural products which make them attractive for the bioenergy sector. Additionally, *Ulva lactuca* contains a higher percentage of carbohydrates compared to wheat, which are the present substrate for ethanol production. So clearly there is a huge potential for adopting this species for energy production, also because the production of 1 ton of algae takes up about 1.5 ton of CO_2.

The main constituents of macroalgae carbohydrates are sugar polymers of both C5 and C6 sugars. In their mono-saccharide form, they can serve as substrate for production of fuel or so-called bioenergy carriers such as ethanol, butanol and biogas (methane) production. However, most macroalgae also have a significant content of salt due to the fact that they grow in salt waters. The literature on conversion of *Ulva lactuca* biomass to bioenergy carriers is very limited. A few simple studies have focused on production of biogas (methane), and the aim of this project is to make the first deeper steps into the conversion technology. The key for an improved bioconversion is development of efficient pretreatment technologies. No studies have examined high efficient technologies, such as thermal pretreatment, that has been developed for preparation of recalcitrant organic substances found in plant biomass (straw etc.). One limiting factor for biogas production from *Ulva lactuca* biomass is the low C:N content (10-25) (Bruhn et al 2011). The optimal C:N ratio for anaerobic conversion of biomass to methane is approximately 27-32. Lower values can result in ammonia concentrations that might inhibit the process (Kayhanian 1999). Special focus on the C:N content in *Ulva lactuca* is, therefore, important when converting the biomass to biogas. One study has also indicated that a low C:N content in *Ulva* species affects negatively the bioconversion capability (Habig et al. 1984). However, the lignin content in *Ulva* species is less than half of those found in other higher aquatic and terrestrial macrophytes (Habig et al. 1984. To our knowledge only one other study has examined the possibilities of converting biomass from *Ulva lactuca* into bioethanol with poor yields (Isa, 2009). However, the high carbohydrate content of *Ulva lactuca* (approximately 55–60%) makes this chlorophyte an obvious candidate for bioethanol production (Pádua et al. 2004). Pretreatment and enzymatic processes have been studied intensively for conversion of terrestic plant biomass to bioenergy carriers (e.g. Thomsen et al 2010) and a few studies exist on macroalgae (e.g. Kadar and Thomsen 2010). Due to the more complex structure and composition of macroalgae, a whole new research area is foreseen to be explored regarding pretreatment and chemical characterization to get full understanding and methodologies for efficient extraction of sugars and other products.

There are only a few published studies of sustainability assessment of macroalgae-based fuels within the scientific literature. A recent study (Thornley at al 2011) found that macroalgae is a renewable resource that could provide GHG reductions of 84%. A heat load is necessary to maximize GHG reductions and there are considerable uncertainties; yet, economic sustainability is not adequately demonstrated. The feasibility of macroalgae cultivation at the scale required for the biofuel market and its associated costs are uncertain (Roesijadi et al 2010); however, algae contain much protein and

some lipids and can replace high amounts of animal feed, thus externalities in terms of a land-use change are considered to be in the other direction than usual. Key drivers are energy security, greenhouse gas reductions, minimizing land-take, and it should be remembered that all energy utilization has impacts (both positive and negative) (Thornley at al 2011). Positive externalities in terms of increased quality aquatic environment and climate mitigation, favor a good cost-effective environmental balance, which is a prerequisite for future sustainable societies. Key questions relating to the use of macroalgae for production of green energy and energy carriers are centered on 1) where and how it can be produced and 2) the economic feasibility of this production and its conversion to liquid or gaseous fuels i.e. ethanol and butanol or biogas.

In the present study, we have investigated technical solutions for *Ulva lactuca* for biomass production and end-use at power plants. This has not been done before. By combining the specific knowledge of each participating partner in this project, integrated technological solutions are illustrated and evaluated for production of energy and energy carriers (i.e. solid biofuel, ethanol, butanol and biogas from the macroalgae *Ulva lactuca)*. In addition, *Ulva lactuca* has been considered and tested as a solid biofuel for combustion and for co-combustion with other solid fuels. One important aspect was to investigate the possibility for reduction and assimilation of CO_2 from power plants for biomass production followed by energy conversion technologies. Thus, the results also describe and evaluate production facilities for *Ulva lactuca* for utilization and CO_2 uptake from power plants including preliminary recommendations for methods of CO_2 transfer from flue gases, mass production of algae biomass and transformation of algae biomass into bioethanol, butanol, biogas and solid biofuel. In terms of sustainability aspects and greenhouse gasses consideration, N_2O emission was studied during growth and production of *Ulva lactuca*. The results from the present study and recommendations will be used for establishment of new production facilities for future generation of sustainable energy supply from a vast unexploited aquatic biomass source – the macroalgae.

1.2 Overview

In this project, methods for producing liquid, gaseous and solid biofuel from the marine macroalgae *Ulva lactuca* has been studied. To get an understanding of the growth conditions of *Ulva lactuca*, laboratory scale growth experiments describing N, P, and CO_2 uptake and possible N_2O and CH_4 production are carried out. The macroalgae have been converted to bioethanol and methane (biogas) in laboratory processes. Further the potential of using the algae as a solid combustible biofuel is studied. Harvest and conditioning procedures are described together with the potential of integrating macroalgae production at a power plant.
The project focuses on the following research tasks:

- N, P and CO_2 capture by *Ulva lactuca* cultivated in basins
- Dry matter production of *Ulva lactuca* cultivated in basins
- Utilization of CO_2 from flue gas by growth of *Ulva lactuca*
- Production of N_2O and CH_4 from *Ulva lactuca*
- Characterization of *Ulva lactuca* biomass
- Conversion of *Ulva lactuca* biomass to bioethanol and butanol

- Conversion of *Ulva lactuca* to methane (biogas)
- Utilization of biogas residuals and ash as fertilizer
- Harvest technology and conditioning for combustion and gasification
- Physical and chemical analyses of *Ulva lactuca*
- Cost calculation of dry and wet *Ulva lactuca* as a biomass resource
- Evaluation of the use of dry *Ulva lactuca* as a fuel in power plants
- Evaluation of *Ulva lactuca* production as fuel in a power plant.

1.3 Overall Conclusions

1. Annual yield of *Ulva lactuca* is 4-5 times land-based energy crops.
2. Potential for increased growth rate when bubbling with flue gas is up to 20%.
3. Ethanol/butanol can be produced from pretreated *Ulva* of C6 and – for butanol – also C5 sugars. Fermentation inhibitors can possibly be removed by mechanical pressing. The ethanol production is 0,14 gram pr gram dry *Ulva lactuca*. The butanol production is lower.
4. Methane yields of *Ulva* are at a level between cow manure and energy crops.
5. Fast pyrolysis produces algae oil which contains 78 % of the energy content of the biomass.
6. Catalytic supercritical water gasification of *Ulva lactuca* is feasible and a methane rich gas can be obtained.
7. Thermal conversion of *Ulva* is possible with special equipment as low temperature gasification and grate firing.
8. Co-firing of *Ulva* with coal in power plants is limited due to high ash content.
9. Production of *Ulva* only for energy purposes at power plants is too costly.
10. N_2O emission has been observed in lab scale, but not in pilot scale production.
11. Analyses of ash from *Ulva lactuca* indicates it as a source for high value fertilizers.
12. Co-digestion of *Ulva lactuca* together with cattle manure did not alter the overall fertilization value of the digested cattle manure alone.

1.4 Suggested Future Perspective for Macroalgae

1. Large-scale production of macroalgae must be off-shore due to large area requirements.
2. New productions methods must be developed in order to lower the costs.
3. A biorefinery concept is needed to extract high value products as proteins, food and feed ingredients, materials, etc. before end use for energy.
4. Macroalgae is the new biomass resource for the next decades.

1.5 Executive Summary

This project has from the very beginning had a very high attention among the medias, both newspapers, radio, TV, technical magazines and at conferences. It has been easy to get an abstract accepted for both national and international conferences, because the project idea is new and the macroalgae is a not researched biomass resource for large scale applications. In addition there is an imagination both in the public opinion and among descision makers that macroalgae is a vast, unexploited biomass resource which can be useful in the future to replace products based on fossil fuels.

The list of publications and disseminations is long. It is presented in Annex 1, Annex 7 and Annex 9. Reward is given to 2 posters for outstanding layout and high technical level. There is listed a total of 67 dissemination activities spread over the following key areas:

- 8 scientific papers published or in preparation. Annex 1
- 26 oral presentations on national and international conferences. Annex 1
- 13 posters on national and international conferences. Annex 1 and 9
- 19 interviews and articles in TV, radio, magazines and newspapers. Annex 1
- 1 conference 12.-13 October 2011 with international speakers. Annex 7

1.5.1 Summary of Ulva lactuca Production

U. lactuca was cultivated in an open pond outdoor system during most of a full growth season (April to September). The cultures were aerated continuously and added minor concentrations of liquid mineral fertiliser. Biomass densities ranging from 1 to 16 kg FW m^{-2} were tested, indicating that maximal growth rates were achieved the lower the biomass density, whereas the biomass yield was maximised at a biomass density of 4 kg m^{-2}. Sporadic sporulation was observed, possibly due to water temperatures exceeding 20 ºC, but the phenomenon did not pose a major problem. A potential areal biomass yield of 45 T DW ha^{-1} $year^{-1}$was estimated on the basis of the results.

Laboratory studies with addition of CO_2 and flue gas to cultures of *Ulva lactuca* indicated that addition of CO_2/flue gas has the potential to increase growth rates by up to 20%. Two flue gas sources were applied, deriving from combustion of wood pellets and a 85/15 mixture of coal/straw, respectively. Addition of two types of flue gas as alternative to chemically clean CO_2 did not disqualify the biomass for any utilisation regarding concentrations of heavy metals.

1.5.2 Summary of Conversion of Ulva lactuca to Bioethanol and Butanol

Characterization of *Ulva lactuca* showed slightly different results found in the literature. The applied methods were able to analyse the sample, however further improvements are necessary in order to complete the mass balance. For analytical determinations samples should be cleaned carefully from sand and other contaminants, like shells. Pretreatments (hydrothermal and wet oxidation) on *Ulva lactuca* did not improve the enzymatic convertibility.

Experiments on the enzymatic hydrolysis of *Ulva lactuca* showed no significant difference in final glucose concentrations between pretreated and untreated biomass. This is likely because cellulose and hemicelluloses are already freely accessible by the enzyme mixtures and quantities used.

Ethanol production using *S. cerevisiae* on hydrolyzed *Ulva lactuca* shows similar to slightly higher yields than obtained by Isa et al. (2009). In addition to glucose, *S. cerevisiae* is able to metabolize fructose produced by enzymatic hydrolysis. Rhamnose is not consumed during fermentation however, leaving a potential carbon source available for further processing of the waste stream. *Ulva lactuca* could be used as a raw material for second generation bio-ethanol production even without pretreatment: Every gram of dry *Ulva lactuca* is converted to 0.141 gram of ethanol in the highest yield

scenario observed during this research. Clostridia cultures grown using hydrolyzed *Ulva lactuca* as a carbon source show low acetone, ethanol and butanol production. Compare to ethanol fermentation studies only 0.065 g butanol/ g dry *Ulva* was achieved. This value decreased even further to 0.050 g/g when pelletized algae was used as a substrate. It is possible this is due to inhibitors present in the macroalgae; however there is no evidence to support this and further research would be required.

1.5.3 Summary of Conversion of Ulva lactuca to Methane

Ulva lactuca can rather easily be converted to biogas. However, in its raw form the organic methane yield (approximately 180 ml gVS-1) and weight specific methane yield (11-12 ml g-1) is rather modest. Simple maceration can make a significant improvement (> 50%) of the organic methane yield while auger pressing or drying improves the weight specific yield (4-7 times). The results of the reactor experiments clearly illustrated that co-digestion of cattle manure and dry *Ulva lactuca* is possible and that the performance of an anaerobic digester treating cattle manure can be significantly improved by addition of *Ulva lactuca*. However, an upper methane production limit of approximately 15-16 ml CH_4 g feed-1 was also observed, which at the current time seems too little for obtaining an economic feasible production at a Danish centralized biogas plant. However, it should be mentioned that despite the low methane yields of *Ulva lactuca* the total methane potential of *Ulva lactuca* equals or exceeds the potential of many terrestrial energy crops due to a fast growth rate.

1.5.4 Summary of Conversion of Ulva lactuca by Pyrolysis and Hydrothermal Treatment

The thermal conversion of algae using traditional methods such as combustion could be challenging considering high moisture and ash content of the algae. Thus alternative conversion methods were tested in order to get first ideas on alternative routes.

The first method was trying fast pyrolysis which turns the major part of the biomass into a liquid, “bio-oil”. This oil could be used for chemicals production or as a liquid fuel which typically recovers 40 – 80 % of the biomass energy content. Tests were made with *Ulva* from the project by CHEC, at the Technical University of Denmark (see Annex 3) in the pyrolysis centrifugal reactor (PCR) and compared with other solid biomass fuels in the temperature range of 550 - 575°C and a total test time of 60-80 minutes. The results revealed that regarding the algae sample, the organic oil yield is 39 wt%, while the algae oil contains 78 % of the feedstock energy content. This gives a promising way to upgrade algae to liquid fuel with high energy recovery efficiency. Due to the low temperatures during pyrolysis compared to combustion, the method is not significantly disturbed by the high ash content of algae. The biomass should however be dried prior to the conversion. Consequently, another method that could process the algae wet would be beneficiary.

A method that could convert the biomass in a wet state is methane production by catalytic supercritical water gasification (SCWG). Such method has been developed at the Paul Scherrer Institute (PSI), Switzerland, (see Annex 4) which agreed on doing some initial test using *Ulva* from the project in their test set up. The gasification of *Ulva Lactuca* was performed at supercritical water conditions (400 °C, ~ 30 MPa) for 60 min over 2 wt.% Ru/C and the results gave an indication that the catalytic

supercritical water gasification of *Ulva lactuca* is feasible and a methane- rich gas can be obtained. The results also revealed that the gasification behaviour of the macroalgae is similar to the ones observed with other algae biomass in previous tests carried out at PSI. However, only in a continuously operated plant such as "PSI's hydrothermal process" with integrated salt separator (in order to remove the catalyst poison sulphur) it may be possible to convert *Ulva Lactuca* fully to biomethane without the presence of unreacted carbon in the aqueous phase.

1.5.5 Summary of Production of N_2O and CH_4 in Ulva lactuca Aquaculture

Algae biomass is a renewable carbon source with potential for energy purposes and greenhouse gas mitigation options. Studies indicate, however, that algae growth may provide a source of greenhouse gases, in particular the potent nitrous oxide (N_2O) having a global warming potential (GWP) approx. 300 times higher than carbon dioxide (CO_2). This study evaluated the net balance of greenhouse gases (GHG) in an algae (*Ulva lactuca*) biomass production system, with focus on N_2O emissions and CO_2 uptake. Measurement campaigns in a pilot-scale growth facility revealed no N_2O emissions. In contrast, under optimal growth conditions significant N_2O emissions, along with CO_2 uptake, were demonstrated from vital *Ulva lactuca*. The N_2O emission depended on the presence of light and availability of nitrate (NO_3^-) in the media. This indicates that N_2O emission from *Ulva lactuca* is not exclusively related to bacterial activity. We hypothesize the presence of an unrecognized nitrate reductase activity associated with *Ulva lactuca* which has not been accounted for before. Applying the concept of global warming potential (GWP), the laboratory data indicates the N_2O emission to account for 0.05-1.3% compared to the CO_2 uptake by the algae.

1.5.6 Summary of Ulva lactuca's Potential as Fertilizer

The objectives of the study were:

- to determine the fertilizer value of the effluents originating from cattle manure co-digested with *Ulva lactuca* in comparison to the anaerobically digested cattle slurry alone
- to investigate the potential greenhouse gas emissions (N_2O, CO_2) after application of the different slurries and
- to obtain information about key soil processes underlying the observed effects.

To achieve these aims, a pot experiment with barley plants and a soil incubation study were set up simultaneously. The co-digestion of *Ulva lactuca* together with cattle manure did not alter the overall fertilization value and GHG emission potential of the digested cattle slurry alone.

1.5.7 Summary of harvest and conditioning of Ulva lactuca

There is extensive experience to harvest aquatic biomass for bioremediation. Among other things, water hyacinths are a big problem in Lake Victoria, where thick mats of water hyacinths cover the lake and cause massive depletion. Also the archipelago of Bohuslän on Sweden's west coast and the Åland islands in the Baltic Sea are particularly plagued by large mats of green algae *Clodophora spp*. and *Enteromorpha spp.* In the Venice Lagoon in Italy large amounts of *Ulva Rigida* are collected each year to reduce the negative impact from the macro algae. When harvest takes place due to bioremediation the macroalgae is normally dumped in landfill and are not used.

Harvesting of macroalgae for industrial use takes place for production of food additives or hydrocolloids in both East Asia, Africa, America and Europe. An example is production of different species of Carragenan. Production systems for warm water species is East Asia and Africa are manual systems and very labour-intensive. Cold water species of Carragenan are produced in America and Europe and are to a minor extent semi-mechanised, but still primitive.

Harvesting of macroalgae for food is well-known in East Asia and Japan. China is far the largest producer of macroalgae for food. According to FAO, the world production of macroalgae for all purposes in 2007 was 14.3 million wet tons. The harvest is in general manual with primitive mechanical equipment.

The studiy of existing harvest technology shows that there are many primitive methods to harvest macroalgae, and all of them are man power intensive. The lack of industrial production systems seems similar to how the fishing sector was developed 100 years ago in Europe with small boats with or without sail. At that time the equipment was simpel nets and lines with hooks. One of the conclusions in this project is that large scale production of macroalgae must take place off shore with modern equipment designed specifically for the purpose and developed from the modern fishing sector. Land-based production systems are needed for special purpose as research, breeding of new species and hatcheries.

In this project harvest took place in June 2009 in Odense Fjord. The *Ulva* was primarily lying at the bottom of the sea as a thick carpet of approximately ½-1 m which made it easy to harvest large amounts rapidly. The harvest was carried out with lawn rakes and the *Ulva* was gathered in vessels before it was placed on europallets to let the excess water drain off before any further transport. 1000 wet kg was harvested. The fresh *Ulva* was washed in 7 different containers containing fresh water to eliminate salt (primarily Na, Cl and K) and other foreign particles, e.g. fauna, from the surface of the *Ulva*. Laboratory tests (Bruhn et al. unpub. data) have shown that it is possible to remove all the salt from the macroalgae surface by thorough cleaning in 7 vessels of fresh water.

Subsequently, the Ulva was pressed mechanically in an auger press making sure that as much water as possible was removed. This process turned out to be extremely suitable for pressing *Ulva*. The pressing was additionally improved because the *Ulva* before pressing was pretreated in a grinder that normally is used for grinding of grass. A mass balance of the wet *Ulva lactuca* processed in the auger press is calculated. The result reveals that the moisture content is only lowered from about 85 % to 72 % by the pressing process. This is far from enough in order to get the *Ulva lactuca* in a storage stable form and further drying is necessary. However, the pressing does significantly separate 1/3 of the ash with the liquid phase and is thus as rather simple and energy-efficient as a first processing step. The main part of the ash removed is soluble NaCl and some Mg , S, Ca and K. This auger press step also turns out to be important for enhancing the conversion efficiency of the downstream processing of the *Ulva lactuca* to biogas and bioethanol. The final drying took place in drying oven at 105°C. Additional analyses were carried out. The dry *Ulva lactuca* was stored in darkness in black bags at room temperature.

The dried *Ulva lactuca* was pressed to pellets in an Amandus KAHL pellet press with a ø6 mm flat die. The dry *Ulva lactuca* is brittle, so milling is not needed. The best result and highest quality of pellets was obtained when the dried *Ulva lactuca* was added water to a total water content of 18-20%. Immediately after the press the water content was approximately 16% and after cooling and initial drying due to rest heat in the pellets from the pelletising process the moisture content was 13-14%. The pelletising of the *Ulva lactuca* is easy and requires less energy than wood pelletising. No binder was used. The quality of the pellets is high with mechanical stability of 99,5%.

1.5.8 Summary of analyses of Ulva lactuca

The composition of *Ulva lactuca* varies depending on where it grows and at what point of the season it is harvested. The analyses do thus only reflect the composition of some representative samples giving an idea on the typical composition of *Ulva lactuca* harvested under Danish conditions. According to the samples investigated the ash content can range from 14 % to 50 % depending on where it is grown, the season and if the biomass has been pretreated or not. This is by far higher than typical solid biomass fuels which are used in power and heating plants today and does thus render some challenges. The highest ash contents in the *Ulva lactuca* samples are found in samples harvested in the sea which are considerably higher than samples harvest from pool trials. This is due to contamination of sand and sea shells clinging to the macroalgae. This explanation becomes obvious when the chemical analyses of the ash composition reveals high amounts of Ca (shell) and Si (sand) compared to the samples from the pool trials. The other cause for the high ash content is high amounts of salts. Some of these salt are remains of the salt water the macroalgae is growing in (high NaCl), but this part can be almost removed by either washing in fresh water or pressing out the water from the algae like in the auger press. The very high amount of salt in ash could cause problems as it would cause problems in thermal conversion units melting ash (slagging and fouling).

However the high amounts of especially K could also make it valuable as fertilizer. Even after pretreatment and removal of surface salts, the remaining ash is still a mixtures of salts containing K, S, Ca, Mg and P. These elements are all important nutrients and if extracted or collected after converting the organic part of the algae, these would serve as a high value fertilizer. Analyses of heavy metals revealed the detectable amounts of Zn and Cu, while all other were below the detection limits of the WDXRF (~10 mg/kg). The detection limits of the WDXRF are unfortunately above the limiting values for heavy metals such as Cd (2.5 mg/kg) in bio ash according to The Danish Bioash Order No. 1636. It can thus not be completely ruled out that this limit is exceeded.

1.5.9 Summary of Ulva lactuca Production at Power Plants

Basins (or raceways) for *Ulva lactuca* production at power plants in the total size of 1 hectare are designed to give an idea of the equipment needed and the costs for investment and operation. 4 basins are designed, each in a size of 2500 m^2 with a length of 100 meter and a width of 25 meter. The basins are made of concrete with a height of 0.6 meter, and the depth of the water is 0.3 meter. The bottom of the basins is flat. The basins are arranged two and two beside each other with a distance of 15 meter to make

piping and transport of macroalgae simple. The circulation of the macroalgae is done by paddlewheel and the speed of the water is 20 cm/sec. The harvest equipment which is a conveyor band is placed at each basin and is submerged during harvest. The speed of the water transports the macroalgae to the harvester. Harvest takes place once a week and the total amount to be harvested is around 400 wet tons annually for 1 hectare basins. The amount per harvest is 8 tons. The macroalgae is transported by conveyor band to a drum drier. Dewatering takes place on the band and the water content is maximum 80% when entering the drum drier. The capacity of the drier must be up to 1000 wet kg/hour drying from 80% to 10% water equal to 7-800 kg evaporation/hour.

In order to obtain high dry matter production capacities in algae production basins, the limitation of CO_2 transport from the atmosphere to the algae basins can be eliminated by supply of flue gas from power plants. Flue gases contain high amounts of CO_2 from combustion of fuels like coal, oil, gas, wood and straw. Another potential concentrated CO_2 source is off-gas from ethanol production plants.

Flue gas from three types of combustion plant gas is considered in this project:
- Coal-fired power plant equipped with deNOx-plant, ash removal system and desulphurisation plant (pulverized fuel)
- Wood chips-fired combined heat and power plant (grate firing)
- Straw fired combined heat and power plant (grate firing).

In the project application it was proposed to distribute flue gas in the algae basins. However, it has now been realized that emission of flue gases from basins close to the ground is environmentally unsafe and will probably not be accepted by the authorities. Collection of gases from the basins is not technical/economically feasible. As an alternative it is proposed to transport salt water from the basins to a flue gas scrubber placed at the power plant.

1.5.10 Summary of Cost Calculations of Ulva lactuca Production at Power Plants
The cost for dry *Ulva lactuca* as solid biofuel is calculated for production facilities at a power plant in the size of 1 hectare raceways (basins) with CO_2 injection and compared to the price of straw production. The costs for wet *Ulva lactuca* for methane production are calculated, and the income for heat and electricity production is compared with the expenditures. The calculations include the following traditional steps:

- Estimation of the capital costs for the basins, buildings and machinery
- Estimation of the operational costs for dry and wet *Ulva lactuca* production
- Estimation of the total cost with CO_2 injection
- Comparison of the prices with similar products for energy production.

The annual costs for the 1 hectare system are 3,717,000 DKK and the income by selling the *Ulva lactuca* as fuel for a power plant is 50,000 DKK which is the price for a similar amount of straw delivered at a power plant. Wet *Ulva lactuca* for a biogas CHP plant can produce 8000 m^3 of methane annually, and this amount of gas can give an income by producing heat and electricity of 40,000 DKK. The annual expenditures are 3,168,000 DKK. It is clear from these calculations that a concept where the only outcome of the system is biomass for energy purposes is far too expensive compared to

the value of the biomass produced. The conclusion is that there must be extraction of high value products from the macroalgae before end use for energy, and the calculated 1 hectare system is far too small; thus there must be designed much larger production systems. A 100 hectares land-based production system will decrease the expenditures remarkably in relation to the production, but the expenditures will still be around 10 times the income.

1.5.11 Summary of Ulva lactuca Use at Power Plants

Co-firing of biomass in coal-fired power plants is a proven technology for CO_2-reduction. The co-firing potential depends on the physical and chemical properties of the biomass product, i.e. moisture content, particle size and content of ash, alkali, chloride and other components. From a combustion point of view dried macroalgae powder is suitable for pulverized fuel co-firing, but the content of ash, alkali, chloride and sulphur is very high. On a heating value basis the content of Ca, Mg, K, Na, S and Cl in *Ulva lactuca* is very high in comparison with coal and also much higher than in straw. By co-firing of *Ulva* in coal-fired power plants the content of Mg, K and Na in the fly ash and the content of SO_2 and HCl in the raw flue gas will be significantly increased. The share of *Ulva* co-firing is limited by the impact on slagging, catalyst deactivation, corrosion, emissions and residue quality (fly ash, bottom ash, gypsum). It is expected that the influence on fly ash quality is the most critical factor and a calculation for 0-20 % co-firing on mass basis has been performed and compared with the critical quality requirements for fly ash used in concrete according to the European standard EN450-1.

The influence on the content of alkali and MgO is substantial and the ash quality standards are exceeded even by 10 % *Ulva* on mass basis, corresponding to 5 % on energy basis. In comparison, by co-firing of 20% straw on mass basis the content of alkali is increased to only 3.6% and there is no significant change in the content of MgO. It is concluded that the use of *Ulva* powder as direct co-firing fuel in coal-fired power plants is very limited.

The limitations mentioned above may however be overcome by new technologies. Low-temperature circulating fluidised bed gasification (LT-CFB) for biomass with high content of ash, alkali and chloride is to be demonstrated in 6 MW-scale. With this technology + 90 % of the ash is separated from the gasified fuel ahead of co-firing and allows high shares of high-alkali biomass to be co-fired in a power plant.

Another possibility is to burn macroalgae on inclined step grates where the fuel is pushed through the combustion chamber by moving grate bars. This type of grate is designed for low quality biomass as household waste, bark or wood chips with high moisture content up to 55-60% water and in addition with a high ash content. The macroalgae can be burned on this type of grate after pretreatment where the water content is reduced from 85% to 60%. This type of grate is built in sizes from 1 MW to 100 MW where 1 MW is the size of farm scale boilers and 100 MW is the size of combined heat and power plants.

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2 Ulva lactuca Production

2.1 Ulva Species – General Description

Ulva is a genus of marine and brackish water green macroalgae. It is edible and often called 'Sea Lettuce'. *Ulva* species have a relatively high growth rate compared to other algae – in nature as well as in cultivation facilities. In nature, growth rates of up to 35% have been reported (Pedersen and Borum 1996). Ulva grows attached to stones or other substrates, but it easily detaches and grows well free floating, often forming dense mats – sometimes even called "green tides" – see Fig 2.1.

Fig. 2.1.a. Ulva lactuca in nature

Fig. 2.1.b. Green tide in China

Ulva has been cultivated successfully for biomass production and bioremediation of waste water from land-based aquaculture (Bruhn et al. 2011; Msuya and Neori 2008; Robertson-Andersson et al. 2008; Ryther et al. 1984). Several cultivation methods are tested and described ranging from different size ponds to different size raceways, with both high and low energy input. *Ulva* is an opportunistic species with a capability to proliferate fast upon fortunate environmental conditions, such as high ambient nutrient concentrations and light. Generally, there is a co-limitation of growth by light and nitrogen (N), meaning that in order to utilize high N concentrations and achieve high growth rates, incoming irradiance must be high (Lapointe and Tenore 1981). When not limited by availability of nutrients, yields are directly correlated to incoming light with higher irradiance supporting a higher areal biomass density (Bruhn et al. 2011). Various species of *Ulva* are reported to be able to utilize carbon (C) in the form of CO_2 as well as in the form of HCO_3^- (Gao and Mckinley 1994). Regarding pH, dissolved inorganic carbon (DIC) and CO_2, the growth rate of *Ulva* is documented to decline at pH values above 7.5-8. In mass cultures of *Ulva*, sporulation may occur in response to seasonal environmental cues and reduce the biomass of vegetative thalli by more than half within a few days.

2.2 Growth Experiments

In this project, cultivation experiments with *Ulva lactuca* were carried out from May to September 2008 in a land-based facility at the Danish Shellfish Centre, Nykøbing Mors (Fig. 2.2). The cultivation tanks had a surface area of 1 m^2 and a water depth of 60 cm.

Natural sunlight supplied the sole input of light with a daily dose in the range of 7 to 59 mol photons m^{-2} d^{-1} and a mean ± SD of 38.7 ± 12.9 mol photons m^{-2} d^{-1}. Unfiltered, untreated surface water from the adjacent estuary (Limfjorden) was continuously supplied during daytime at a rate of 5 l min^{-1}. Salinity ranged between 25 to 28.5 ‰ and water temperature between 7 and 23°C, respectively, during the experimental period. Nutrients were added to the cultivation tanks continuously over two hours every evening (8 to 10 pm), to approximate concentrations of 15 µM of nitrate-N (NO_3^--N), 5 µM of NH_4^+-N and 2.5 µM ortho-phosphate (ortho-P) in the tanks, using a solution of liquid greenhouse fertilizer (Blaakorn Drivhusgoedning, Bauer, Germany). The nutrient additions corresponded to loadings of 0.17 g dissolved inorganic nitrogen (DIN) m^{-2} d^{-1} and 0.048 g dissolved inorganic phosphorus (DIP) m^{-2} d^{-1}. Gentle aeration of the water using a centrifugal blower continuously circulated the algae between surface and bottom of the pond with approximately one minute intervals. Stocking densities of algae were 1, 2, 4, 6 or 8 kg fresh weight (FW) m^{-2}, distributed in triplicate between randomly selected tanks. Algae biomasses were harvested and manually adjusted to initial stocking densities at least once a week. The specific growth rates were calculated from the FW of the algae: SGR = 100 ×[ln (W_t/W_0)]/t, where W_0 corresponded to the initial stocking density, and W_t to the biomass after t days of cultivation.

Fig. 2.2. The cultivation facility at Mors (photo: Michael Bo Rasmussen)
Fig. 2.3. Inspection of the biomass in one of the large tanks (photo: Lars Nikolaisen)

2.2.1 Growth and Production

The biomass yields obtained in this experiment demonstrated that a substantial amount of *Ulva lactuca* biomass can be cultivated at latitudes as far north as Denmark (56° N). Extrapolation of the results obtained in this study leads to an estimated annual biomass production of 45 t dry weight (DW) ha^{-1} y^{-1}. This is 2-20 times the production potential of conventional terrestrial energy crops (McKendry 2002, Lehtomaki et al. 2008) and three times the production of brown algae in temperate waters (Kelly and Dworjanyn 2008) (Fig. 2.4). The highest area of specific biomass yield was achieved with a stocking density of 4 kg FW m^{-2}. The production rate varied considerably during the growth season, mainly due to fluctuations in daily incoming irradiance, and to a lesser extent temperature (Table 2.1 and Fig. 2.5).

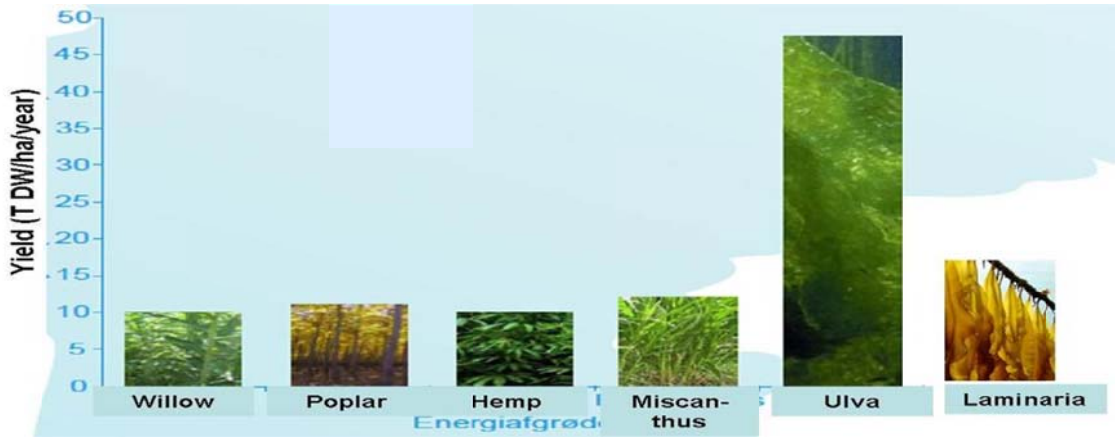

Fig. 2.4. Comparison between the annual yield of some land-based and marine energy crops (T DW per ha per year)

The presented annual production estimate is based on the correlation between average incoming light and biomass production for this stocking density, assuming a seven month growth season (mid-March to mid-October) and using a 30 year average of daily incoming light. Our results are in agreement with the findings of other studies, (i.e. the US Aquatic Species programme), where larger production rates have been generated in energy-intensive production systems, whereas lower rates were documented in non-energy-intensive systems, 74 versus 26.7 T DW ha^{-1} y^{-1} (Ryther et al, 1984). The daily biomass yields and growth rates found here are in range with a maximal yield of 55 g TS m^{-2} d^{-1} and maximal specific growth rates of 18% d^{-1} reported from studies with integration of *Ulva lactuca* in multitrophic aquaculture using similar size tanks and using a stocking density of 1 kg FW m^{-2} (Neori et al. 1991), but lower than the biomass yields reported under cultivation of *Ulva lactuca* with high nitrogen loads: 37.6 ± 8.6 g TS m^{-2} d^{-1} (mean ± SD) reported by Msuya and Neori (2008) (calculated from the FW:TS ratio reported in the reference).

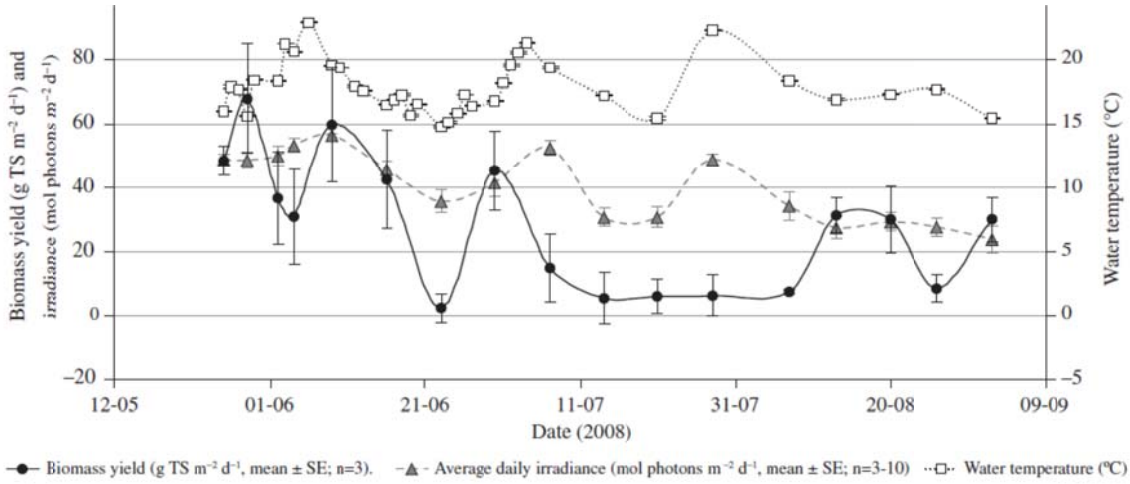

Fig. 2.5. Biomass production (●) (gTSm^{-2} d^{-1}, mean ± SE; n = 3) in tanks with a biomass density of 4 kg FWm^{-2}, average daily irradiance (○) (mol photons m^{-2} d^{-1}, mean ± SE; n = 3–10) and water temperature (▲) (ºC, mean ± SE; n = 3) as a function of time. The low biomass yield recorded on the 23rd of June was the result of sporadic sporulation events in some of the tanks (Bruhn, et al, 2011).

Table 2.1. Biomass yields (g TS m^{-2} day^{-1}; mean ± SE; n = 3). Highest biomass yield in every period is marked in bold (Bruhn, et al, 2011).

Experimental period (date of harvest, 2008)	Biomass density (kg FW m^{-2})				
	1	2	4	6	8
1 (26.05)	43.8 ± 4.8		**48.5 ± 4.6**		−3.1 ± 15.7
2 (29.05)	9.7 ± 5.2		**67.9 ± 17.0**		−1.7 ± 14.5
3 (02.06)	16.6 ± 5.0		**36.8 ± 14.5**		26.0 ± 13.7
4 (04.06)	16.6 ± 11.3		31.0 ± 15.0		**39.8 ± 36.7**
4 (09.06)	26.1 ± 3.4		**59.7 ± 17.8**	12.9 ± 6.9	
5 (16.06)	32.7 ± 3.6		**42.7 ± 15.6**	29.3 ± 7.1	
6 (23.06)		**27.3** ± 4.9	2.4 ± 4.5		
7 (30.06)		41.0 ± 7.3	**45.4 ± 12.2**		
8 (07.07)		**17.4 ± 8.0**	14.9 ± 1.05		
Average (26.05–07.07)	24.5 ± 3.5	28.5 ± 4.9	**38.8 ± 5.4**	21.1 ± 5.7	15.2 ± 9.4

2.2.2 Biochemical Composition

The biochemical composition of the harvested and cultivated algae has been analysed. One interesting parameter is the carbon to nitrogen (C:N) ratio of the harvested biomass, since this represents a way of estimating the internal allocation of energy to carbohydrates (C) versus proteins (N). Also the concentration of monosaccarides was analysed, as well as the content of metals in the biomass from the flue gas experiments.

C:N ratio: The C:N ratio of the cultivated algae ranged from 7.9 to 24.4 and was positively correlated to incoming irradiance (R^2= 0.54, $p < 0.01$.). Likewise, a clear correlation was observed between irradiance and the concentration of monosaccharides in the algae $R^2 = 0.40$, $p < 0.05$ (Fig. 2.6).

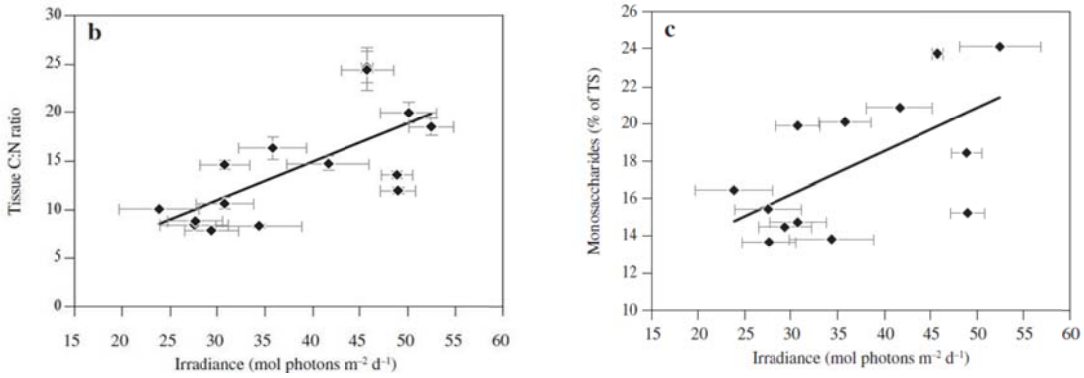

Fig 2.6. The correlation between average daily irradiance and the C:N ratio (mean ± SE, n = 3) of the Ulva lactuca biomass and Fig. 2.2.6 the monosaccharide content (% of TS. mean ± SE) of the Ulva lactuca biomass (mean ± SE (Bruhn, et al 2011)).

This is in accordance with several previous findings showing that macroalgae tend to accumulate carbon, and therefore in many cases also carbohydrates, when growing at light levels above their light saturation point (Chapman and Lindley 1980, Markager and Sand-Jensen 1992, 1994 and 1996). Habig et al (1984) found a higher content of soluble carbohydrates and neutral fibres, and a lower content of protein and crude fibres, in nitrogen starved *Ulva lactuca* (C:N ratio of 30.71). Nitrogen starved *Ulva lactuca* biomass proved superior to nitrogen-replete biomass regarding production of methane. In this study, the increasing C:N ratio with increasing light levels is probably

not indicating nitrogen limitations, but rather that carbon fixation exceeds the maximum growth rate for the algae resulting in a temporary storage of carbohydrates. We observed that the internal N pools are only occasionally below the critical value of 2.17% of TS reported as limiting for maximal growth (N_C). The N pools are never near or below the subsistence quota (N_Q) of 0.71% of TS (Pedersen and Borum 1996). This has important implications for the optimal strategy for cultivating algae for bioenergy purposes. Presumably, the best strategy is to maximize light exposure and balance the nutrient addition in order to achieve the optimal balance between overall growth rate and accumulation of carbohydrates. Nitrogen starvation has been described and discussed as a means to increase the C:N ratio and hence, the concentration of carbohydrates of the biomass (Fig. 2.7).

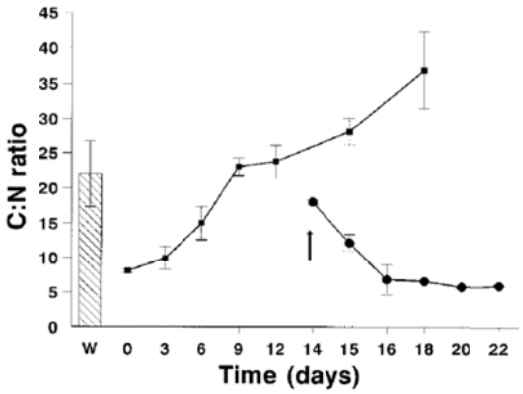

Fig 2.7. Effect of nitrogen starvation (■) and enrichment (●) on the C:N ratio of Ulva rigida. Arrow shows the day of change of nitrogen-starved algae to nitrogen-enriched seawater. Bar shows the C:N value of wild collected U. rigida (Pinchetti et al, 1998).

However, there is a trade-off between growth and carbohydrate storage: Decreasing nitrogen availability will result in slower growth and eventually death of the biomass. Additionally, a low nitrogen concentration in the growth environment increases the risk of the *Ulva* to sporulate and thus the biomass to disintegrate and vanish. Results from cultivating *Ulva lactuca* on a range of nitrogen concentrations (N source was degassed pig manure, N concentration was measured as NH_4^+ concentration), indicate that although an increase in C:N ratio is clearly observed as a consequence of decreasing environmental N concentration, this increase is caused by a decrease in internal N concentration, whereas an increase in internal carbon concentration is not achieved (Table 2.2). As mentioned, a lower N content may still increase the value of the biomass for energy production in the form of biogas (Habig et al, 1984).

Table 2.2. Tissue contents of C, N and P in Ulva lactuca after exposure to different external NH_4^+ concentrations for 10 days. Values indicated are means ± SE (n=3). Values in same row with different lettering differ significantly from each other (ANOVA, p<0.05) (Nielsen et al, submitted).

	6 µM N	12 µM N	25 µM N	50 µM N	100 µM N
C/N	26.02 ± 0.20^a	23.64 ± 0.26^b	18.91 ± 0.67^c	16.15 ± 0.14^d	10.88 ± 0.29^e
C (% of DW)	37.0 ± 1.43^a	35.8 ± 0.72^a	36.5 ± 0.37^a	36.8 ± 0.56^a	38.13 ± 1.22^a
N (% of DW)	1.66 ± 0.07^a	1.77 ± 0.05^a	2.25 ± 0.09^b	2.66 ± 0.04^b	4.09 ± 0.14^c
P (% of DW)	0.084 ± 0.004^a	0.115 ± 0.001^b	0.144 ± 0.005^c	0.279 ± 0.007^d	0.397 ± 0.006^e

2.3 CO_2 Concentrations and Quality

A number of experiments with different concentrations of CO_2-enriched air and pH control were carried out as pilot experiments prior to the actual flue gas experiments. The pilot experiments were carried out in the laboratory at AU, Department of Bioscience in Silkeborg. The flue gas experiments were carried out in a mobile lab at the actual site of the flue gas emission. Flue gas is not decompressable, and hence not transportable, since the gas changes characteristics upon decompression, mainly due to condensation of water and following dissolution of particles and compounds such as dust, NOx and sulphur. The results from the pilot experiments indicated that pH was to be kept between 7 and 8 for optimal growth rates.

2.4 *Ulva lactuca* Response to Flue Gas

CO_2-enrichment has been documented to increase the growth rates of *Ulva lactuca*. However, flue gas as a source of CO_2 contains other compounds than chemically clean CO_2, i.e. sulphur, NOx, dust particles and various metals. It is important to clarify the potential effect of these substances on the growth of *Ulva lactuca*. Reports regarding green microalgae and a red macroalgae demonstrate that there is no difference between the effects of flue gas and CO_2 on growth rates – and no critical metal concentrations, if the flue gas was cleaned (Israel 2005. Dostouva 2009).

In this project, we have documented that cleaned flue gas has the same effect on the biomass production of *Ulva lactuca* and no negative effects on biochemistry. We tested the effect of flue gas from two different sources (wood pellets and coal/straw) on the growth rates, C:N ratio and metal concentrations of *Ulva lactuca*. Experiments were carried out at Danish Technological Institute in Aarhus, and at the Studstrup power plant, respectively (Fig. 2.8). Experiments were carried out in laboratory scale comparing the effect on algae growth and biochemical composition of three treatments:

1. flue gas from combustion of wood pellets or coal/straw
2. air enriched with CO_2 and O_2 in flue gas ratio (13%/6%)
3. atmospheric air.

The addition of flue gas and CO_2-enriched air was controlled by the pH of the medium.

Figure 2.8.a Experiments with cultivation of Ulva lactuca on real flue gas were carried out on-site, inside the chimney at the Studstrup power plant. 2.8.b The Studstrup power plant

Results showed that addition of flue gas as well as CO_2-enriched air increased the growth rate by up to 21% compared to atmospheric air. As growth is exponential, if the cultures are not limited by the availability of nutrients or carbon, such an increase in growth rates can result in a substantial areal biomass yield (Fig. 2.9). However, the surplus yield does strongly depend on the intervals for harvesting the biomass.

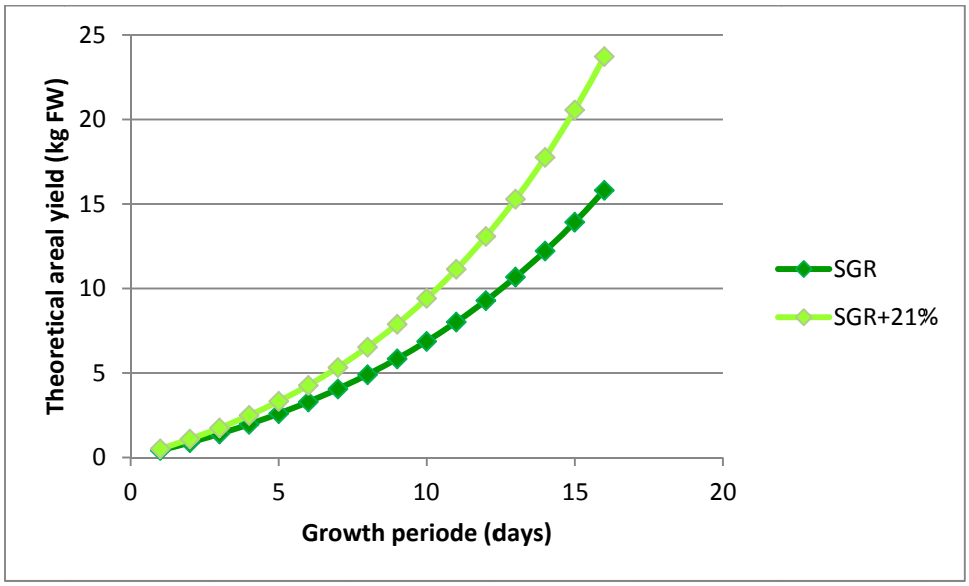

Figure 2.9. Illustration of the potential increase in biomass yield achieved by increasing growth rate by 21%. In this example, a biomass density of 4 kg FW m^{-2} and growth rates (SGR) of 10% day^{-1} and 12.1% day^{-1}, respectively, is used. As can be observed, the theoretical gain depends on the harvest intervals applied.

The nitrogen content of the produced biomass was significantly higher in the flue gas and CO_2 treatments, whereas no effect of the flue gas was detected on the concentrations of heavy metals (Table 2.3), or on the monosaccharide composition of the biomass. There was no significant difference between the effect of flue gas and CO_2-enriched air on growth and biochemical composition. We conclude that there is a potential for simultaneous CO_2 bioremediation and increased sustainable biomass production by controlled flue gas addition to cultivation of *Ulva lactuca*.

Table 2.3. Metal concentrations in Ulva cultivated with different C sources. (Mean ±SE, n=3).

Metal	Flue gas (ppm)	CO_2 enriched air (ppm)	Atmospheric air (ppm)	Limit values agriculture[a] (ppm)	Limit values feed[b] (ppm)
Arsen (total)	*1.65±0.16*	*2.25±0.59*	*0.82±0.06*	*25*	*3*[c]
Cupper	*8.72±0.12*	*9.64±1.53*	*15.95±1.50*	*1000*	-
Zink	*30.30±1.82*	*32.27±1.00*	*30.58±2.98*	*4000*	-
Crome	*0.72±0.13*	*0.51±0.05*	*0.61±0.04*	*400*	-
Nickel	*1.15±0.12*	*0.92±0.09*	*1.60±0.18*	*30*	-
Lead	*0.50±0.08*	*0.29±0.05*	*0.49±0.04*	*120*	*5*
Mercury[d]	*0.22±0.12*	*0.22±0.04*	*0.13±0.17*	*0.8*	*0.1*
Cadmium	*0.04±0.01*	*0.02±0.002*	*0.05±0.01*	*0.8*	*0.5*

[a] *http://www.dmu.dk/foralle/jord/slam_i_landbrugsjord/tungmetaller_i_slam/*
[b] *According to French legislation on edible seaweeds (Beseda et al, 2009)*
[c] *Inorganic arsen. Not total arsen.*
[d] *Caution when using biomass for food/feed*

2.5 Production of N_2O and CH_4 in Ulva Lactuca Aquaculture

2.5.1 Introduction

Nitrous oxide (N_2O) and carbon dioxide (CO_2) are atmospheric trace gases, which directly and indirectly influence the current and future global climate (Forster et al. 2007). The global warming potential (GWP) of N_2O is 296 times higher than equal mass of CO_2 and N_2O is currently responsible for approx. 10% of the global climate forcing. Sustainability assessment of new energy technologies thus has to take into consideration not only the CO_2 saving potentials, but also detrimental emissions of other greenhouse gases. Biogenic production of N_2O from bacterial nitrification and denitrification taking place under reduced oxygen conditions are considered the major pathways for N_2O emissions (Kroeze *et al.* 1999). Significant N_2O emissions have been measured from N-enriched rivers, estuarine and coastal water, as well as freshwater lakes, reservoirs and wetlands receiving a high N load (Bange 2006; Suntharalingam and Samiento 2000; Mengis *et al.* 1997; Seitzinger and Kroeze 1998; Seitzinger *et al.* 2000; Groffman *et al.* 2000; Silvan *et al.* 2002; Huttunen *et al.* 2003a, b; Wang *et al.* 2007). Littoral zones, the transitional boundary between terrestrial and aquatic ecosystems, have been suggested as potential hotspots of N_2O production during algal blooms (Groffman *et al.* 2000; Wang et *al.*, 2006, 2007).

Whereas N_2O production associated with bacteria living in the water column, the sediments, interior of suspended particles and guts of invertebrates is well documented (e.g. Schropp and Schwartz 1983; Nevison *et al.* 2003; Codispoti *et al.* 2005; Stief *et al.* 2009), a seemingly unaccounted contribution might be from the growth of aquatic plants and algae itself. Recent reports demonstrating direct N_2O emissions from terrestrial plant leaves during the plant nitrogen (N) assimilation have emphasized the need to study this hitherto under-investigated potential source (Smart and Bloom, 2001; Hakata *et al.* 2003; Goshima *et al.* 1999). The capability of N_2O emission in plant leaves has been attributed to the nitrite (NO_2^-) reduction (NiR) pathway, and a key role for NiR in producing the N_2O has been demonstrated in wheat (Smart and Bloom,

2001). The NiR enzyme is also found in algae chloroplast (Solomonsen and Barber 1990; Crawford 1990; Ferrario-Méry *et al.* 1997), yet production of N_2O in macroalgae is not clearly documented. To our knowledge there is only one report on direct N_2O emission from living algae (Weathers 1984), which shows that axenic cultures of algae Chlorophyceae (*Chlorella*, *Scenedesmus*, *Coelastum* and *Chlorococcum*) produced N_2O when NO_2^- was available in the growth media, and when environmental conditions were in favour of photosynthesis to occur. Another potential source of N_2O associated to the growth of algae is by epiphyton, i.e. microorganisms attached to the algae surface (Law et al. 1993). Marine Cyanobacteria has been reported to produce N_2O from NO_2^- (Weathers and Niedzielski 1986), and it can be speculated if the presence of Cyanobacteria on the surface of macroalgae can be a source of "algae-derived" N_2O. Mengis et al. (1997) concluded, that while under oxic conditions bacterial denitrification is unlikely as possible N_2O sources, the production of N_2O in the oxic epilimnion in lakes must be attributed to active growing algae.

In this context, further studies are needed to elucidate possible mechanisms and conditions for N_2O production associated with extensive growth of macroalgae. This information is a prerequisite for thorough assessment of the sustainability of biomass production systems based on growth of macroalgae.

We investigated N_2O emissions and photosynthetic uptake of CO_2 in the macroalgae *Ulva lactuca*, which is considered significant for biomass production. We hypothesized that:

1) the presence of N_2O emissions will depend on nitrite availability in the growth media, and
2) algae-derived N_2O emission will depend on photosynthetic activity.

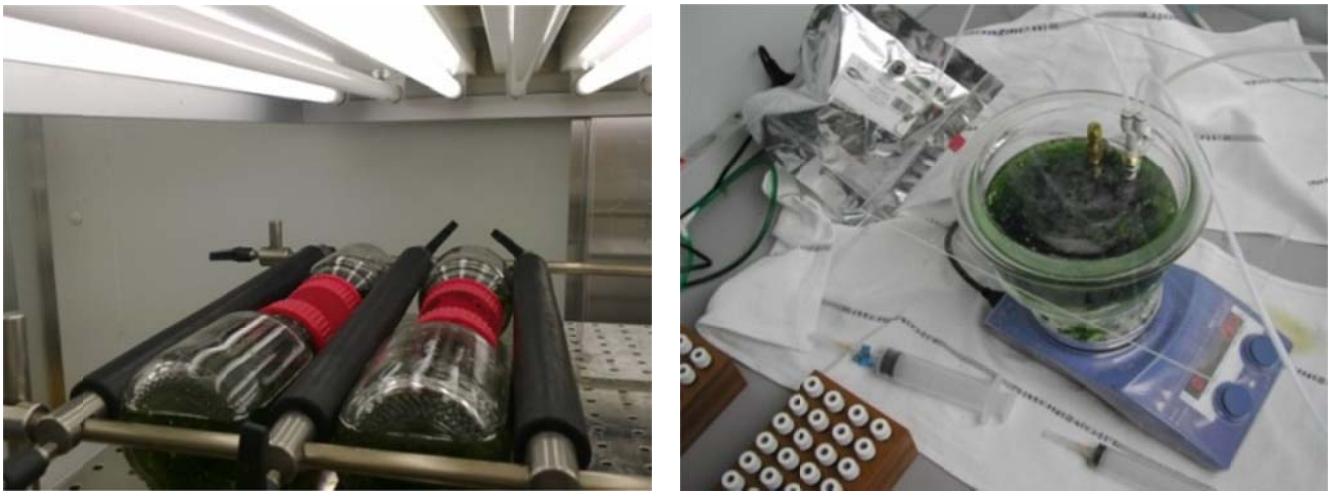

Fig. 2.10 Experimental setup for laboratory incubations of capped static bottles (left hand picture) and flow-through incubator (right hand panel). The laboratory incubations were conducted with different combinations of media, presence of Ulva lactuca biomass and light.

2.5.2 Experimental Setup

In the current study, we investigated the potentials for N_2O emissions from *Ulva lactuca* under two environmental conditions, i.e. (*i*) controlled laboratory conditions to address the factors controlling N_2O, and (*ii*) pilot-scale growth facilities.

Laboratory study (*i*): a series of laboratory experiments were conducted with *Ulva lactuca* cultures under different combinations of N substrates (NO_2^-, NO_3^- at ca. 1.6-3.2 mM g^{-1} fw) (Weathers 1984) and light conditions (darkness or 225 µmol m^{-2} sec^{-1}). The courses of N_2O and CO_2 gas exchange were measured in closed vessels using Photo Acoustic Detection and conventional Gas Chromatography (GC) analysis. We used free floating *Ulva lactuca* collected in the bays Roskilde Fjord and Hjarbæk Fjord. The algae were kept in culture for 6-8 weeks and were then replaced by new batch. Only vital material with active CO_2 uptake was used for incubations. All media were prepared using filtered (2µm) seawater. Stocks were autoclaved at 121°C for 20 minutes and kept in dark cooler (5°C) until use. The incubations took place in (1) 250 ml closed cap static bottles where headspace samples were removed for gas analysis, and (2) in larger (2.l0) incubator with a continuous flow-through of headspace gas (Fig. 2.10).

Pilot-scale study (ii): The investigations on greenhouse gas emissions under pilot-scale growth conditions have been applied at two campaigns to the test facility at "AlgeCenter Danmark" in Grenaa in October 2010 and August 2011. The emissions were measured by deployment of floating gas flux chambers (40 x 40 x 15 cm) to the larger reservoir (Fig. 2.5.2), or by removing a sub-sample for incubation in a smaller stand-alone incubator. The latter was achieved in order to avoid the vigorous air-bubbling of the growth basins, which impeded gas flux measurements. Nutrients were added to the basins at rates of 50 µmol NO_3^--N g^{-1} FW d^{-1} (2010) and 40 µmol NO_3^--N g^{-1} FW d^{-1} (2011).

Fig. 2.11 Deployment of floating gas flux chamber to pilot-scale growth basin at the test facility at "AlgeCenter Danmark", October 2010, Grenaa

2.5.3 Results and discussion

Laboratory study (i): No N_2O emissions were observed from capped bottles incubated in light or in dark with pure media, neither from seawater or seawater plus nitrate nor from seawater plus nitrite. Dark incubations including algae showed no N_2O emissions in any of the media combinations. Light incubations including algae showed no N_2O emissions in media seawater, but significant N_2O emissions from seawater plus nitrite and seawater plus nitrate. In batches from Roskilde Fjord the emissions in presence of nitrate averaged (±SE) 0.22±0.03 µg N_2O-N hr^{-1} DW^{-1} (n=24); batches from Hjarbæk Fjord averaged 0.11±0.01 µg N_2O-N hr^{-1} DW^{-1} (n=12).

The N_2O and CO_2 dynamics during growth of *Ulva lactuca* were further verified from real-time measurements in flow-through incubator (Fig. 2.12). During initial conditions in light there were no emissions of CO_2 or N_2O from seawater plus nitrate alone (Phase 1). By removing the lid, the measured CO_2 level increased due to mixing with the higher CO_2 concentration in the laboratory (Phase 2). Upon addition of *Ulva lactuca* and resealing the incubator, photosynthesis exponentially reduced the headspace CO_2 level along with a linear increase in the N_2O emission (Phase 3).

The rate of headspace CO_2 sequestration in the series of experiments averaged 6.9±0.6 mg $_{CO2}$-C hr^{-1} g DW^{-1}; The N_2O emissions averaged 0.02±<0.01 µg N_2O-N hr^{-1} g DW^{-1} (N=8). Considering a N_2O GWP of 296, this suggests that N_2O emission counterbalanced on average 0.07% of the CO_2 sequestration in the incubator experiments. Assuming a similar carbon sequestration per g DW in the capped bottle incubations, then the N_2O emissions counterbalanced from 0.24% to 1.32% of the CO_2 sequestration.

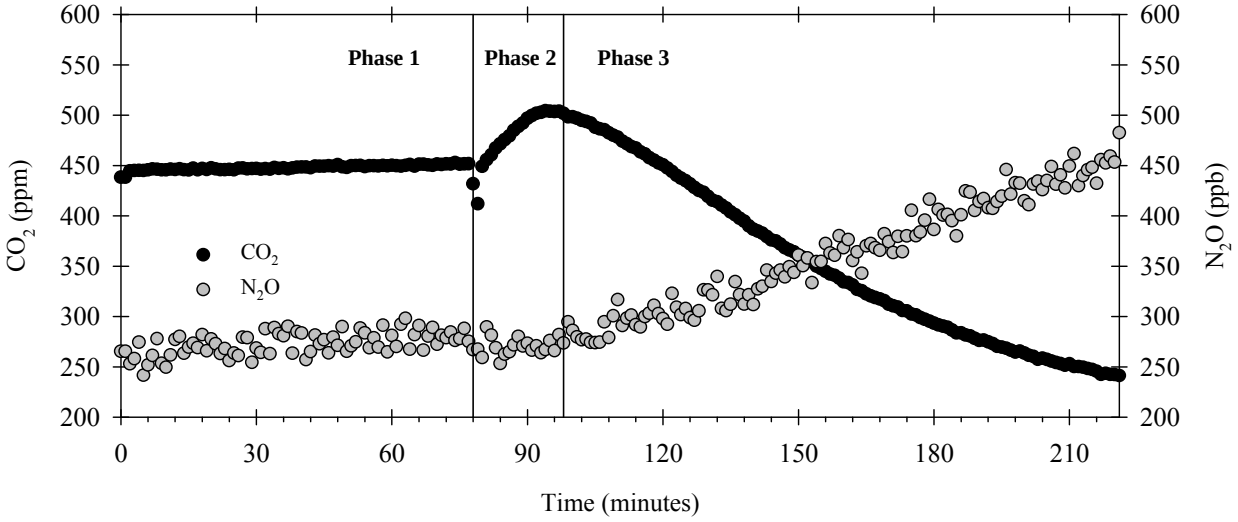

Fig. 2.12 Dynamics of N_2O (grey symbols) and CO_2 (dark symbols) in headspace of flow-through incubator prior to addition of Ulva lactuca biomass (phase 1), during opening of the incubator (phase 2), and algae photosynthetic after sealing the incubator (Phase 3).

Pilot-scale study (*ii*): In the October 2010 study, no N_2O emissions were observed using floating chambers in the growth basins (Fig. 2.13). We consider whether the lack of

activity was due to relatively cool temperatures, poor light conditions and limited substrate availability. In August 2011 sub-samples of media + algae were harvested from the growth basins and incubated under *in situ* conditions; as in the first campaign, no N_2O emissions were observed, indicating that substrate N limited the N_2O. The harvested biomass from both campaigns subsequently demonstrated N_2O emissions under optimal growth conditions in the laboratory. It cannot be ruled out, that optimized growth of *Ulva lactuca* in large-scale facilities may induce conditions where N_2O emissions will take place, in particular under conditions when e.g. extensive recycling of wastes (manure; sewage) is practiced to provide a source of nutrients. Therefore *in situ* measurement techniques of N_2O may need more development to scale up the emission balance to growth conditions.

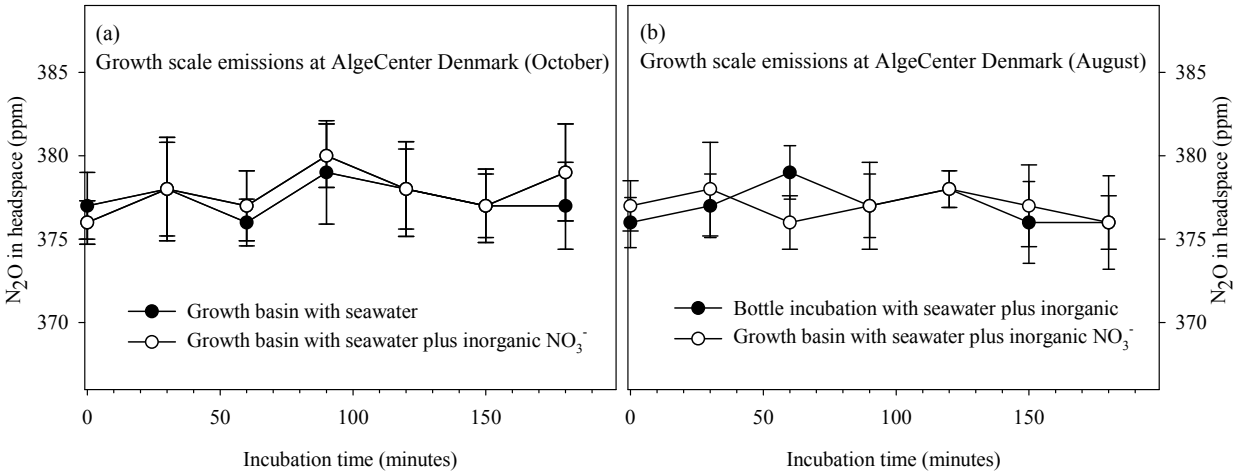

Fig. 2.13 N_2O emissions from the growth scale facility AlgeCenter Danmark. (a) Measurements conducted in 2010 from the head space of floating, transparent chambers when Ulva lactuca was grown in seawater only (closed symbol) or in seawater with added NO_3^- (n=4±stdev). (b) Measurements conducted in 2011: subsamples of Ulva lactuca transferred to stand-alone incubator (51 l capped bottle; n=6; closed symbols) or floating chambers (open symbols; n=2).

Generally, the series of experiments does not rule out the possibility of microbial processes mediating N_2O emission rather than the algae *per se*. However, the absence of N_2O emission in darkness, supporting our hypothesis (2), suggests that algae photosynthetic activity plays a key role in the production and emission of N_2O. One speculation could be that the photosynthetic production of labile carbon compounds serves as substrate for bacterial activity. In support to our hypothesis (1), N_2O production did occur when NO_2^- was available in the media; but we also discovered a significant emission of N_2O when NO_3^- was added as sole N source. It could be considered that the apparent relationship between NO_3^- and N_2O production in *Ulva lactuca* is mediated by an unrecognized nitrate reductase enzyme associated to *Ulva lactuca*.

The initial objective of the current task was to study not only N_2O production but also production of methane (CH_4) in aquacultures of *Ulva lactuca*; however, preliminary analysis indicated no activities concerning CH_4, and it was decided to focus on the very potent greenhouse gas N_2O. In addition to CH_4, other volatile organic carbon compounds (NMVOC) that have consequences for atmospheric chemistry might also be emitted from algae (Sartin et al. 2001). In a first draft of the project description we

mentioned to include NMVOCs in the study; however, due to budget cuts, this task was later abandoned and removed from the task description (*Revision 2009.03.26*); unfortunately the mentioning of NMVOC was never removed from the project "table of contents", and thus it also appears in the current report.

2.5.4 Concluding Remarks

Emissions of N_2O occurred from *Ulva lactuca* during growth under optimal laboratory conditions with NO_2^- or NO_3^- as N-nutrients and in the presence of light. This possible source of N_2O is almost completely overlooked in the literature budgeting greenhouse gas balances in aquatic systems. Our laboratory data suggest the *Ulva lactuca* N_2O emission to counterbalance 0.05% to 1.3% of the CO_2 sequestration observed in light, in terms of global warming potentials. Short-term campaigns in pilot-scale growth basins did not reveal any emissions of N_2O. Thus, emissions of the potent greenhouse gas N_2O does not seem prohibitive for a sustainable production of *Ulva lactuca* biomass. Future studies across a larger diversity of algae families and under various environmental conditions are needed to screen for the occurrence and variability of this potential important process.

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3 Conversion of Ulva lactuca to Bioethanol and Methane

3.1 Characterization of Ulva lactuca Biomass and Residuals following Bioconversion

In order to obtain the most appropriate strategy for the conversion of the seaweed to bioenergy, a proper characterization of the biomass was performed. Next to the subject of this project two other species of macro algae were tested: Different *Ulva lactuca* (Jutland, Denmark, Fig. 3.1), *Chaetomorpha linum*, and *Gracilaria longissima*, from Orbetello lagoon, located in southern Tuscany, Italy. The algae were analyzed by means of strong acid hydrolysis for contents of cellulose, hemicellulose, starch, lignin, extractives and ash. The results are given in the table below (Table 3.1).

Fig. 3.1 Fresh Ulva lactuca samples (Jutland, Denmark)

Table 3.1 Composition of different macroalgae

component	*U. lactuca (1)*	*U. lactuca* (2)	*U. lactuca* (3)	*U. lactuca* (4)	*G. longissima*	*C. linum*
glucan	6.1	7.1	5.3	6.7	20.1	29.9
cellulose	4.7				12.9	26.3
starch	1.4				7.2	3.6
hemicellulose	4.1	3.5	2.6	2.6	21.2	9.2
xylan	4,1	3.5	2.6	2.6	14.1	3.2
arabinan	0.0	0.0	0.0	0.0	7.1	6.0
rhamnan	NA	3.5	3.0	2.9	NA	NA
lignin	9.1	8.1	10.7	11.8	24.3	5.3
ash	34.0	29.1	5.8	5.7	7.1	13.4
extractives	15.8*	40.6	57.3	54.1	23.3*	37.0*
SUM	**67.8**	**92.0**	**84.8**	**83.9**	**88.8**	**91,2**

- *U. lactuca* (1): harvested in 2008 (Denmark); *U. lactuca* (2): harvested in 2009 (Denmark); *U. lactuca* (3): harvested in 2010, grown without fertilizer (Denmark); *U. lactuca* (4): harvested in 2010, grown with fertilizer (Denmark); *G. longissima* and *C. linum* (1): harvested in 2008 (Italy)
- *NA: not analyzed*
- *extraction was carried out for 24h with ethanol; in all other cases extraction was carried out by water for 6h, followed by ethanol for 6h.

Total sugar content of *Ulva lactuca* was between 7.2 and 10.9 g/100 g DM (dry matter). Lignin and ash contents varied between 8.1 and 11.8 g/100 g and 5.7 and 34 g/100 g DM, respectively. High ash contents could be an indication for contamination with non-organic material such as sand or sturdy organic minerals such as seashells.

These results show lower sugar concentrations than determined by Isa et al. (2009) for *Ulvales spp*. They found compositions of 15-18 g/100 g DM glucose, 3-4 g/100 g DM xylose and 10-12 g/100 g DM rhamnose. Another study, performed by Al-Amoudi et al. (2009) shows a slightly lower total sugar content of 6.8 g/100 g DM. Carbohydrate contents as high as 58.4 g/100 g sample have been reported however (Pádua et al. 2004), proving that there is a large variation in composition of *Ulva lactuca* species. This can likely be attributed to the different growth and harvest conditions and variation in species of the algae used in this research and other studies.

3.2 Conversion of Ulva lactuca Biomass to Bioethanol

3.2.1 Pretreatment Studies on Macroalgae

Dried and milled samples of *Ulva lactuca*, *G. longissima* and *C. linum* were treated hydrothermally using a stirred and heated reactor with 6% DM/L water at conditions seen in Table 3.2, where composition of raw and pretreated materials are also compared.

Table 3.2 Composition of untreated and pretreated macroalgae

Name	T(°C)	Time (min)	Gas Pressure (bar)	Cellulose (%)	Hemicellulose (%)	Lignin (%)	Starch (%)
U. untr.	-	-	-	4.7	2.8	0	1.4
U. No ox	195	10	N_2 /4	8.6	1.1	0	0.8
U. Ox	195	10	O_2/12	9.9	0.4	0	0.1
G. untr.	-	-	-	12.9	21.2	24.3	7.2
G. No ox	195	10	N_2/4	32.2	4.0	29.8	0.5
G. ox	195	10	O_2/12	29.1	9.2	24.8	0.1
C. untr.	-	-	-	26.3	3.2	6.0	3.6
C. No ox	195	10	N_2/4	39.5	0.8	4,2	8
C. Ox	195	10	O_2/12	67.4	0.5	8.1	0.2

U: *Ulva lactuca* (1), G. *Gracilaria longissima*, C: *Chaetomorpha linum* (1)
untr.: untreated samples; No ox.: pretreated with nitrogen; Ox.: pretreated with oxygen

Pretreatment of fibres resulted in enriched cellulose content and show very good effect on hemicellulose removal (Table 3.2). The maximal recovery of carbohydrates is an important point of an optimal pre-treatment. Both cellulose and hemicellulose recovery were low (< 60% and 10% respectively) at *Ulva lactuca* and *G. longissima*, while the very high cellulose recovery (> 100%) for *C. linum* after pretreatment partly can be explained by its starch content (8%).

Enzymatic hydrolysis was carried out on raw and pretreated materials to test the convertibility of cellulose and starch. Untreated and pretreated samples were hydrolyzed by commercial enzyme preparations (Celluclast 1.5L, Novozym 188, Spirizyme Plus Tech from Novozyme, Denmark), and results are shown in Figure 3.2 (Coppola et al., 2008, 2009; Nielsen et al., 2009).

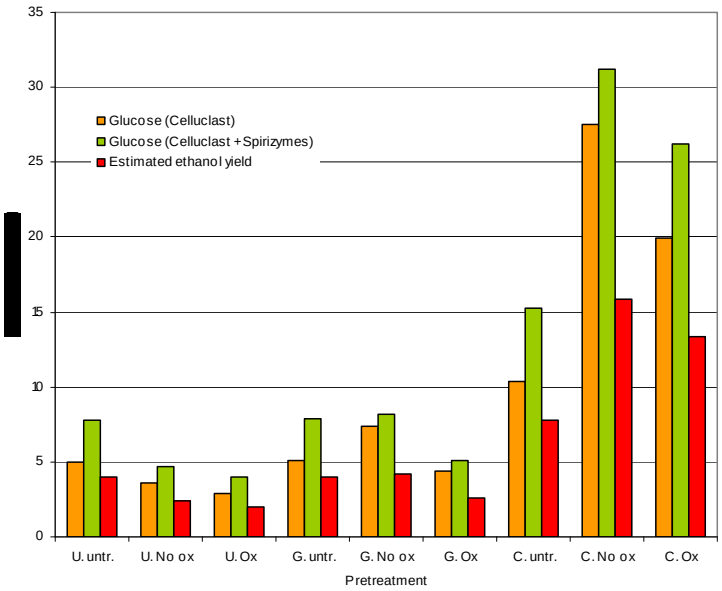

Fig. 3.2. Yield of glucose and ethanol potentials (g/100g DM) after pretreatment and enzymatic hydrolysis.
U: Ulva lactuca; G: G. longissima; C: C. linum
Untr: untreated
No ox: hydrothermal treatment at 195°C, 10 minutes incubation time, 4 bar N_2
Ox: wet oxidation at 195°C, 10 minutes incubation time, 12 bar O_2

Both pretreatments (hydrothermal and wet oxidation) have a positive effect on *C. linum* (Fig. 3.2). The pretreatments have either no effect (hydrtothermal pretreatment) or reduce the ethanol potential (wet oxidation) for *G. longissima*, while both pretreatments reduce the ethanol potential for *Ulva lactuca* (U). Therefore, optimization of pretreatment on *Ulva lactuca* was not carried out.

3.2.2. Ethanol Fermentation Studies

Ethanol fermentation studies were carried out on sterilized (121°C, 20 min) *Ulva lactuca* as a less sever pretreatment method. Small scale Simultaneous Saccharification and Fermentation (SSF) experiments showed similar results as presented in Figure3.2: Untreated algae resulted in higher final ethanol concentration than untreated one..

Therefore, untreated *Ulva lactuca* was used in further SSF studies. Further studies on enzymatic hydrolysis show that yield can be further increased if Liquozyme (α-amylase) is involved in a two steps prehydrolyzis process. Figure 3.3 shows the enzymatic hydrolyzis results of *Ulva lactuca* with different enzyme cocktails.

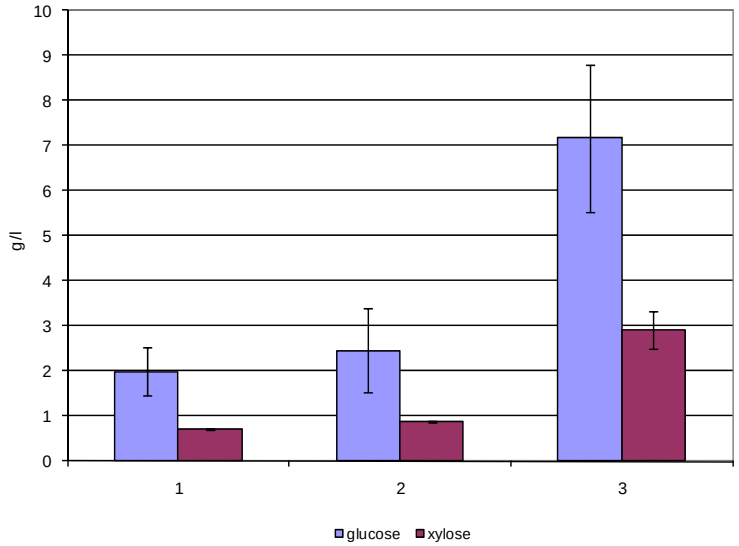

Fig. 3.3. Enzymatic hydrolyzsi of Ulva lactuca by different enzyme cocktails
1, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM (Hydrolysis at 50°C pH4.8)
2, Cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme (Hydrolysis at 50°C pH4.8)
3, Liquozyme and cellulases (Celluclast + Novozyme 188) at 25 FPU/g DM and Spirizyme (Hydrolysis at 85°C for 1h at pH5.7 followed by additional cellulases and Spirizyme at 50°C, pH 4.8).

The highest final glucose content (7 g/l) was achieved when pretreated macroalgae were hydrolyzed by Liquozyme (α-amylase) at 85°C for 1h at pH 5.7 followed by hydrolyzis at 50°C, pH 4.8 applying Celluclast, Novozym 188 and Spirizyme.

For ethanol fermentation studies non pretreated *Ulva lactuca* was hydrolyzed at a concentration of 100 g/l by enzyme mixtures shown above. Hydrolysate was either separated or non-separated (total) from the solid residue and fermented by *S. cerevisiae*. Control experiments on glucose medium were performed with similar initial glucoe content. Gas production was measured during fermentation as the weight loss and final concentrations of ethanol were determined by HPLC after 45 hours of fermentation. A summary of sugar consumption and ethanol production is given in Table 3.3.

Table 3.3 Sugar consumption, ethanol and lactic acid production and ethanol yield of fermentation of hydrolyzed Ulva lactuca by S. cerevisiae.

	Consumed glucose (g/l)	**Produced ethanol (g/l)**		**Produced lactic acid (g/l)**		**Yield (g/g glucose)**	**Yield (%)**
Control	19.48	8.76	*±0.17*	0.20	*±0.02*	0.449	88.13
Total	19.44	12.47	*±1.07*	2.72	*±0.17*	0.641	125.75
Separated	19.19	14.13	*±0.23*	1.78	*±0.08*	0.736	144.40

Yield is calculated based on consumed glucose.

All initial glucose present at the beginning of the fermentation was consumed. Very high yields indicate the presence of other fermentable carbohydrates. The highest ethanol yield detected results in a production of 0.141 g ethanol/g DM *Ulva lactuca*.

3.2.3 ABE (Acetone Butanol Ethanol) Fermentation Studies on Macroalgae

Butanol as a liquid biofuel can provide more benefits than ethanol, due to its gasoline-like properties. It can be produced from the same feedstocks as ethanol (starch and cellulosic sugars) but the butanol producing *Clostridia* species is able to ferment different kinds of carbohydrates including C6 and C5 sugars. The aim of our studies was to test *Ulva lactuca* as possible substrate for ABE fermentation (Kádár et al., 2010, 2011). Two different strains were selected and ordered from the German Collection of Microorganisms and Cell Cultures (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)): *Clostridium acetobutylicum* DSMZ 792 and *Clostridium beijerinckii* DSMZ 6422. Strains were propagated and stock precultures were placed at -85°C.

An analytical method has been established in our laboratory. High performance liquid chromatography (HPLC) equipped with a Shodex KC-811 column can be used to detect both intermediate (acetic, lactic and butyric acids) and final (acetone, butanol, ethanol (ABE)) products. First experiments were performed on synthetic medium, which contained glucose, xylose and arabinose at 50 g/l initial concentration, respectively. Fermentations were carried out at 35°C under anaerobic conditions. *C. beijerinckii* was performing better on synthetic medium, so this has been chosen for further studies.

Studies aimed to test ABE fermentation on pretreated (hydrothermal and sterilization (121°C, 20 min) as a pretreatment method) on dried *Ulva lactuca*. Enzymatic hydrolysis was performed with enzyme mixtures, according to our earlier studies described above. The hydrolysate was further used for ABE fermentation (*C. beijerinckii* under anaerobic conditions at 35°C) with additional glucose to reach the initial 30 g/l glucose content and compare to fermentation on synthetic medium as control (C). Results are shown in Figure 3.4.

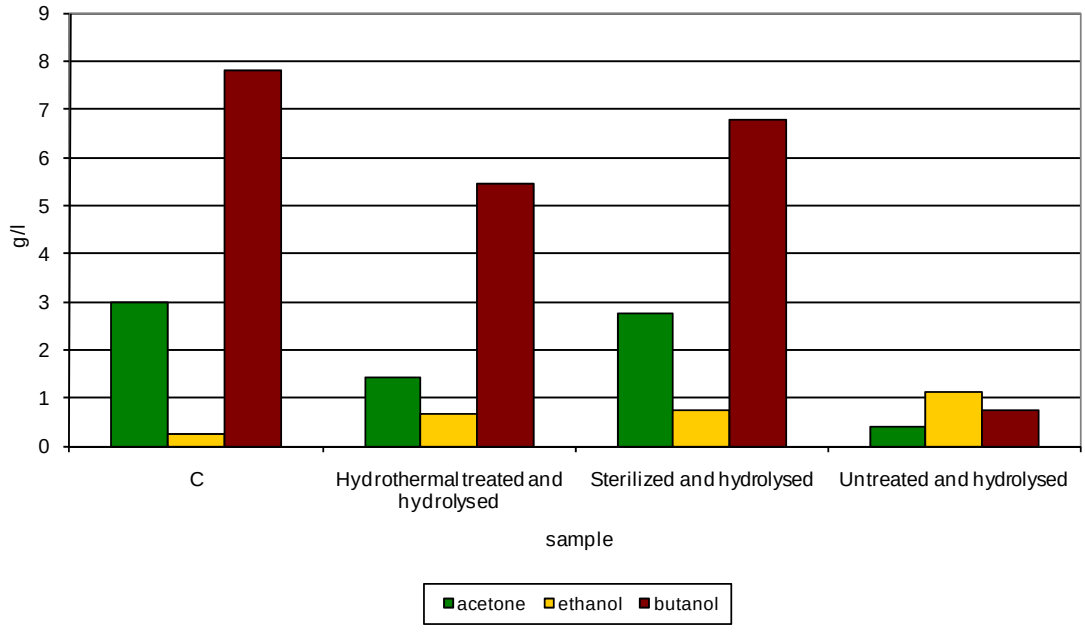

Fig. 3.4 Produced acetone, butanol and ethanol from pretreated and untreated Ulva lactuca. The figure shows the effects on different types of pretreatment

The highest solvent production was achieved when *Ulva lactuca* was pretreated by sterilization. Compared to Control experiment (C) both pretreated sample produced less solvents, which can be indication for some kind of inhibition. That can be studied in the future. Interestingly, compared to ethanol fermentation, untreated and hydrolyzed macroalgae was no suitable substrate for ABE fermentation.

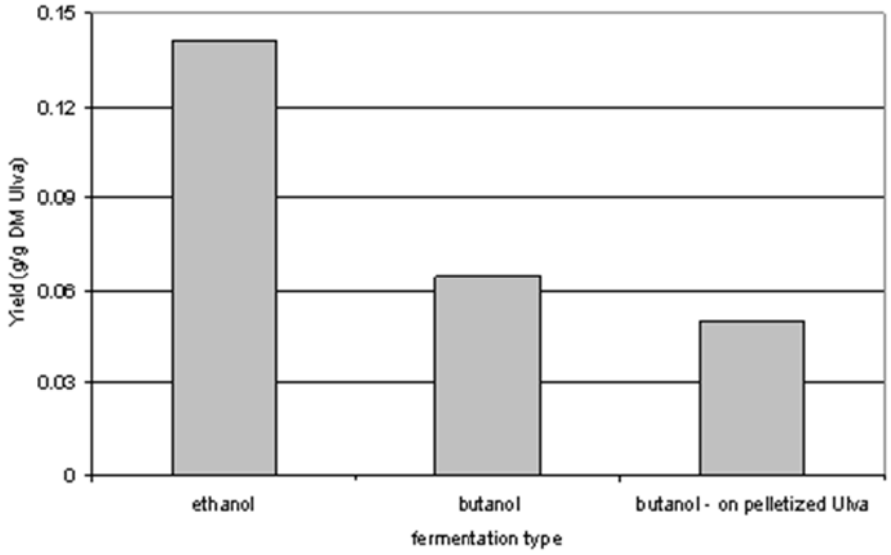

Fig. 3.5 Final ethanol and butanol yields (g product/ g DM Ulva) obtained from Ulva lactuca

3.2.4 Concluding Remarks

Characterization of *Ulva lactuca* showed slightly different results found in the literature. The applied methods were able to analyze the sample; however, further improvements are necessary in order to complete the mass balance. For analytical determinations samples should be cleaned carefully from sand and other contaminants, like shells. Pretreatments (hydrothermal and wet oxidation) on *Ulva lactuca* did not improve enzymatic convertibility.

Experiments on the enzymatic hydrolysis of *Ulva lactuca* showed no significant difference in final glucose concentrations between pretreated and untreated biomass. This is likely because cellulose and hemicelluloses are already freely accessible by the enzyme mixtures and quantities used.

Ethanol production using *S. cerevisiae* on hydrolyzed *Ulva lactuca* shows similar to slightly higher yields than obtained by Isa et al. (2009). In addition to glucose, *S. cerevisiae* is able to metabolize fructose produced by enzymatic hydrolysis. Rhamnose is not consumed during fermentation however, leaving a potential carbon source available for further processing of the waste stream. *Ulva lactuca* could be used as a raw material for second generation bio-ethanol production even without pretreatment: every gram of dry *Ulva lactuca* is converted to 0.141 gram of ethanol (see figure 3.5) in the highest yield scenario observed during this research.

Clostridia cultures grown using hydrolyzed *U. lactuca* as a carbon source show low acetone, ethanol and butanol production. Compare to ethanol fermentation studies only 0.065 g butanol/ g dry *Ulva* was achieved (see figure 3.5). This value decreased even further to 0.050 g/g when pelletized algae was used as a substrate. It is possible this is due to inhibitors present in the macroalgae; however there is no evidence to support this and further research would be required.

3.3 Production of Methane from Ulva lactuca and from Bioethanol Residues

During the project, many different aspects of anaerobic digestion (AD) of *Ulva lactuca* were studied. The effect on the methane potential of the algae by various pretreatments including washing, mechanical maceration, thermal treatment, drying and solid/liquid separation was tested. The potential inhibition levels of *Ulva lactuca* on the AD process were also evaluated and finally we tested co-digestion of *Ulva lactuca* and cattle manure in a continuously stirred tank reactor system (CSTR) with special focus on optimal algae to manure ratio.

3.3.1 Methane Potential of Ulva Lactuca and Effect of Pretreatments (batch systems)

Ulva lactuca was sampled at Seden Beach (Odense Fjord) and frozen at -20^{o}C.

Experiment 1
The algae were exposed to different treatments resulting in eight different batch series (Fig. 3.6). Batch 1 served as control. Here the algae were only roughly chopped (≈ 2 x 2 cm) to facilitate the distribution of the algae in the batch vials. Batch 2 was a homogenized paste obtained by maceration of the algae. Batch 3 and 4 were made by washing the algae in order to dilute the concentration of salts and to remove sand and gravel. 200 grams of algae were suspended in 10 litres of water for 24 hours. After washing, the batch 3 substrate was roughly chopped as in batch 1, and in batch 4 the algae were macerated as in batch 2. In batch 5 and 6 the algae were treated as in batch 3 and subsequently exposed to thermal treatment at 110^{o}C/20 min. and 130^{o}C/20 min., respectively. For preparation of batch 7, the algae were dried at 45^{o}C until a constant weight was obtained. The substrate was subsequently grounded (< 1 mm). Batch 1-7 were digested in 500 ml batch bottles at an algae concentration of 5 gVS l^{-1} (volatile solids) with inoculum from a lab-scale reactor treating cattle manure (5.4 % VS) at a temperature of 52-53^{o}C. In batch 8, the algae were treated as in batch 1 but digested at mesophilic (37ºC) temperatures instead of at thermophilic temperatures with effluent from Hashøj biogas plant.

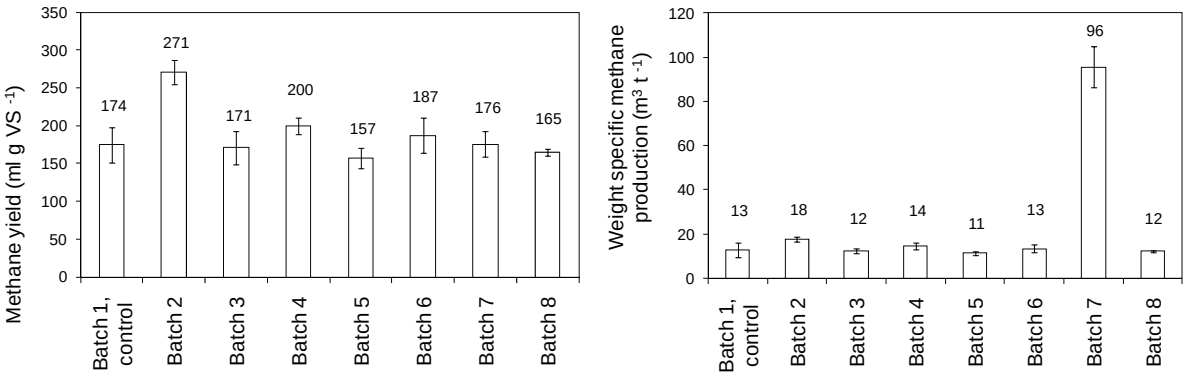

Fig. 3.6 Screening of different pretreatment methods and the effect on the methane yield of Ulva lactuca. Simple batch digestion of Ulva lactuca in bottles performed by addition of digested cattle manure. Before digestion Ulva lactuca was pretreated in the following way: Batch 1 algae were chopped (≈ 2 x 2 cm) to facilitate the distribution of the algae in the batch vials. Batch 2 homogenization with kitchen blender. Batch 3 and 4 washing of the algae in tap water and subsequently chopping (as batch 1) and homogenizing (as batch 2). In batch 5 and 6, the algae were treated as in batch 3 and subsequently exposed to thermal treatment at 110°C/20 min. and 130°C/20 min., respectively. Batch 7 dried and grounded. Batch 8 mesophilic digestion (batch 1 algae) instead of thermophilic digestion.

Washing had no effect on the methane yield as illustrated in the figure 3.6 (batch 3 compared to batch 1). Maceration of unwashed algae resulted in a significant boost (56%) in methane yield from 174 ml g VS^{-1} (batch 1) to 271 ml g VS^{-1} (batch 2). A more moderate increase (17%) as a consequence of the maceration was observed for washed algae (batch 4 compared to batch 3). Thermal treatment at 110°C (batch 5) had a negative effect on the methane yield and treatment at 130°C (batch 6) only gave a 7% increase. The methane yield of the dried algae (batch 7) was in the same range as for the wet algae (batch 1). A decrease of the digestion temperature from 52°C to 37°C (batch 8) lowered the final methane yield with 7%. In general, all yields were relatively low and comparable to e.g. manure.

Various terrestrial energy crops such as maize and grass-clover have higher yields than *Ulva lactuca* (Table 3.4) but the total methane potential (m^3 ha^{-1}) of *Ulva lactuca* is considerably higher than for many terrestrial energy crops when taking the high growth yield of the algae into account. In addition to this, optimized biochemical composition of *Ulva lactuca* via manipulation of light and nitrogen conditions during growth can increase the methane yield and the methane potential of *Ulva species* has been estimated to be between 400-421 l CH_4 g VS^{-1} based on the chemical composition (Habig et al. 1984, Briand and Morand 1997). Development of efficient pre-treatment methods to exploit the full potential of *Ulva lactuca* and make anaerobic digestion more favourable seems necessary.

Table 3.4 Methane potential of selected macroalgae and boreal energy crops and crop residues and organic wastes. As written, Ulva lactuca has a total biogas potential per hectare that exceeds several energy crops although the yield per ton dry weight (TS) is lower.

Substrate	Growth yield ($t\ TS\ ha^{-1}\ y^{-1}$)	Methane yield		Methane potential ($m^3\ ha^{-1}$)
		($m^3\ t\ TS^{-1}$)	($m^3\ t\ VS^{-1}$)	
Macroalgae				
Ulva lactuca	45 [a]	93-155 [a]	162-271 [a]	4200-7000
Ulva energy intensive	74 [b]	-	220-330	-
Ulva non-energy intensive	27 [b]			
Gracilaria	-	-	280-400 [c]	-
Sargassum	-	-	120-190 [d]	-
Sargassum	-		260-380 [e]	-
Laminaria[a]	15 [f]	-	260-280 [e]	-
Macrocystis	-	-	390-410 [e]	-
Crops and crop residues				
Timothy clover grass[b]	8-11 [g]	306 [g]	333 [g]	2600-3600 [g]
Vetch-oat mixture[b]	5-7 [g]	329 [g]	365 [g]	1600-2300 [g]
Jerusalem artichoke[b]	9-16 [g]	306 [g]	333 [g]	2800-4900 [g]
Tops of sugar beet[b]	3-5 [g]	255 [g]	299 [g]	700-1300 [g]
Maize	16 [h]	407 [h]	427 [h]	6500 [h]
Maize	9-18 [i]	-	-	4000-8000 [i]
Straw, wheat	7 [j]			-
Miscanthus	12-30 [j]	-	-	-
SCRC Willow	10-15 [j]	-	-	-
Wastes				
Flotation sludge	-	-	540 [k]	-
Fish oil	-	-	600-800 [k]	-
Meat and bone flour	-	-	570 [k]	-
House hold waste	-	-	400 [k]	-

[a] *Saccharina latissima*
[b] 50 days incubation

[a] *This study;* [b] Ryther et al, 1984 ; [c] Habig et al. 1984; [d] Chynoweth et al. 2001; [e] Chynoweth 2005; [f] Kelly and Dworjanyn, 2008; [g] Lehtomäki et al., 2008; [h] Oleskowicz-Popiel et al.; [i] Seppälä et al. (2008); [j] McKendry, 2002; [k] Angelidaki and Ellegaard 2003.

Experiment 2

Solid/liquid separation. As seen in Experiment 1, drying of *Ulva lactuca* did not affect the methane yield (ml g VS^{-1}) but did improve the weight specific yield (ml g $algae^{-1}$) more than 7 times. This result is important because such concentration of biomass potentially allows a higher organic loading of a continuously fed reactor system and thereby also a higher specific biogas production. Removal of water is also important for the storage stability of the biomass since water speeds up the decay. However, one of the drawbacks of drying is the energy demands making the process expensive. Therefore we tested auger pressing of the algae as a method for solid/liquid separation and estimated the methane potential of the different fractions. The AD of the algae was performed as described in Experiment 1 and the results are given in Table 3.5.

Table 3.5 Methane potential of different Ulva lactuca fractions. By removal of water via auger pressing, the weight-based methane production can be improved 4 times.

Substrate	Methane yield (ml /gVS)	Total solids (%)	Volatile solids (% ww)	Methane production (ml/g substrate)	Volumetric ratio (inoculum /substrate)
Fresh	196 ± 13.7	8.6 ± 0.22	5.8 ± 0.17	11.3	6
Solid fraction	192 ± 3.0	27.4 ± 0.38	22.9 ± 0.29	43.9	23
Liquid fraction	22.8± 16.2	2.6 ± 0.02	0.5 ± 0.01	1.16	0.5

The methane yield of the fresh and solid fraction was 196 ml gVS^{-1} and 192 ml gVS^{-1}, respectively. These yields are well within the range found in Experiment 1. However, auger pressing increased the weight specific yield of the solid fraction approximately four times from 11.3 to 43.9. The yields of the liquid fraction were very low. Due to the low organic matter content of the liquid fraction (0.51% VS), a larger sample volume was applied in the batches, resulting in a low volumetric inoculum to substrate ratio (I/S ratio) of 0.5 and thereby in a higher fraction of seawater. Presumably, the higher salt concentration in the mixture caused the inhibition, as anaerobic digestion is generally sensitive to the salt content (Gourdon et al. 1989).

Experiment 3

Comparison of *Ulva lactuca* with other algae species suitable for cultivation in Danish waters. Samples of *Chaetomorpha linum* (green algae), *Saccharina latissima* (brown algae, previously the genus of *Laminaria*), *Gracillaria vermiculophylla* (red algae) and *Ulva lactuca* (green algae) were gently rinsed in tap water to remove sand and gravel. Subsequently, the macroalgae were exposed to two forms of pretreatment: parts of the algae were roughly chopped (≈ 2 x 2 cm) to facilitate the distribution of the algae in the batch vials while other parts were mechanically macerated. Methane potentials of the algae were estimated as in experiment 1 but incubation time was a little shorter (34 days versus 42 days).

Rather large deviations in the methane yield of the four examined macroalgae were observed, see Table 3.6, ranging from 132 ml g VS^{-1} for *G. vermicullophylla* to 340 ml g VS^{-1} for *S. latissima*. Taking the TS/VS content into account, even larger deviations were observed for the weight specific methane yield ranging from 9.9 ml g $algae^{-1}$ for *Ulva lactuca* to 66.8 ml g $algae^{-1}$for *S. latissima*. Maceration of the algae resulted in a significant (68%) increase in the methane yield of *Ulva lactuca* from 152 ml g VS^{-1} to 255 ml g VS^{-1}. A more modest increase was observed for *C. linum* (17%) and *G. vermicullophylla* (11%) while maceration of *S. latissima* had no positive impact on the methane yield. When only considering the biomass composition, *S. latissima* seems to be most suitable for anaerobic digestion of the examined species. However, if the algae are cultivated for bioenergy production the total methane output will also depend on the growth rates of the macroalgae and should be taken into account. In Denmark, the growth rate of *Ulva lactuca* under natural conditions exceeds the growth rates of the other examined species. In this project *Ulva lactuca* was cultivated in land-based tanks

with a yield of 45 t TS ha^{-1}, corresponding to a total methane potential of 4200-7000 m^3 ha^{-1}. Growth yield estimation of other macroalgae during cultivation in Denmark has to our knowledge not been published but other studies have reported growth yields of *S. latissima*, *G. Vermiculophylla* and *Ulva lactuca* of 15 t TS ha^{-1}, 87.5 t TS ha^{-1} and 74 t TS ha^{-1}, respectively (Ryther et al. 1984; Kelly and Dworjanyn 2008).

Table 3.6 Methane potentials of different macroalgae that potentially can be cultivated in Danish waters.

Substrate	Pretreatment	TS / VS (%)	Methane yield ml g VS^{-1}	Methane prod. ml g $algae^{-1}$
Batch screening of methane potentials of different macroalgae[a]*:*				
C. linum	Washed, chopped	12.22 / 6.86	166 ± 43.5	11.4 ± 2.98
C. linum	Washed, macerated		195 ± 8.7	13.4 ± 1.46
S. latissima	Washed, chopped	24.02 / 20.07	340 ± 48.0	68.2 ± 9.63
S. latissima	Washed, macerated		333 ± 64.1	66.8 ± 12.87
G. vermiculophylla	Washed, chopped	16.91 / 13.12	132 ± 60.0	17.3 ± 4.88
G. vermiculophylla	Washed, macerated		147 ± 56.3	19.3 ± 7.39
U. lactuca	Washed, chopped	9.03 / 6.47	152 ± 18.7	9.9 ± 1.21
U. lactuca	Washed, macerated		255 ± 47.7	16.5 ± 3.08

[a]34 days of incubation

3.3.2 Inhibition Levels of Ulva lactuca (batch systems)

Toxicity effects of *Ulva lactuca* on the anaerobic digestion process were tested in batch experiments. Raw and dried (45°C until a constant weight was obtained) samples of the algae were distributed in 500 ml serum bottles - and mixed with water and inoculum (as described previously) - in final concentrations of 2.5 g VS l^{-1}, 5 g VS l^{-1}, 10 g VS l^{-1} and 20 g VS l^{-1}. Vials were incubated at 53°C for 34 days. A clear inhibition of methanogenesis was seen during the first seven days of vials containing wet *Ulva lactuca* in concentrations of 20 g VS l^{-1}. Fig. 3.7. Hereafter, the adapted process and the highest methane production rate of all series were observed from days 7-13. The final methane yield in vials with concentrations of 20 g VS l^{-1} was however still lower at day 34. Also vials with concentrations of 10 g VS l-1 was somewhat inhibited within the first 5 days when compared to vials with concentrations of 5.0 g VS l^{-1}. For dry algae biomass no clear tendencies of inhibition were observed, although the yield of vial containing 20 g VS l^{-1} was slightly lower than other vials during the experiment. The cause of the observed inhibition's effects of the wet algae biomass was not investigated further in this experiment but might have been due to increasing salt concentrations, organic overloading and subsequent pH drop or competition between methanogens and sulphate-reducing bacteria due to high sulphur concentrations.

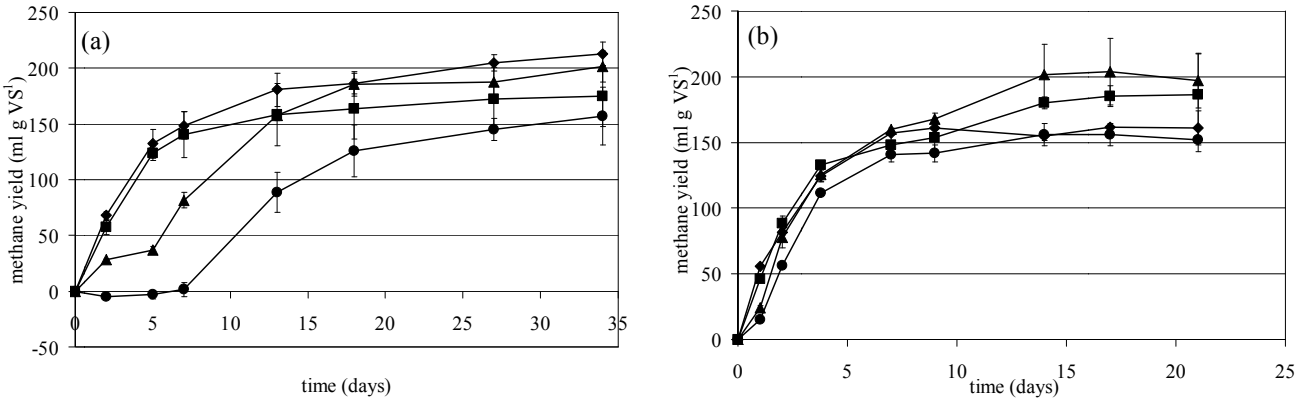

Fig. 3.7 Toxicity tests of Ulva lactuca. Batch vials containing inoculum from a thermophilic CSTR treating cattle manure were added various concentrations (◆: 2.5 g VS l^{-1}; ■: 5.0 g VS l^{-1}; ▲: 10.0 g VS l^{-1}; ●: 20.0 g VS l^{-1}) of wet and dry Ulva lactuca and incubated at 53°C. a) Wet Ulva lactuca b) Dry Ulva lactuca. As seen, the biogas process was more sensitive towards wet algae compared to dry algae.

3.3.3 Co-digestion of Ulva Lactuca and Cattle Manure

Two 4.5 liter CSTR with a working volume of 3.0 liter were inoculated with effluent from a thermophilic full scale biogas plant (Snertinge, Denmark) treating cattle/pig manure (80%) and industrial waste (20%). The reactors (Fig. 3.8) were stirred by a propeller every fifth minute for 45 sec. at 100 rpm and operated at 53°C with a hydraulic retention time (HRT) of approximately 15 days. Initially, both reactors were fed with only cattle manure that had been diluted to a final concentration of 4% VS. The organic loading rate of the reactor (OLR) was ranging from 2.7 g VS $l^{-1}d^{-1}$ during that period. When the reactors had been operating at steady conditions (stable methane production and volatile fatty acids levels (VFA)) for some weeks, the test reactor was supplemented with *Ulva lactuca*.

Fig. 3.8 Experimental setup for co-digestion trial

Dried and grounded algae were added to the feedstock in amounts of 20% of the total VS concentration. As a consequence of this the concentration of the feedstock mixture increased to 4.9% and the OLR increased to 3.3 g VS l^{-1} d-1. After 28 days (app. 2 HRTs) the fraction of *Ulva lactuca* was increased to 40% corresponding to a final VS concentration of the feedstock of 6.6% and an OLR of 4.4 g VS l^{-1} d^{-1}. This loading scheme was kept for 46 days (3 HRTs). Finally the *Ulva lactuca* ratio was increased to

50% corresponding to a feedstock concentration of 7.5% VS and an OLR of 5.0 g VS l^{-1} d^{-1}. This loading scheme was kept for 108 days (7 HRTs). In the reactor experiment (Fig 3.8) we chose to use dry *Ulva lactuca* biomass as a feedstock. The inhibition of dry algae was lower than wet algae. The methane yield of the feedstock showed a slight decrease when 20% *Ulva lactuca* was added and a further decrease during the subsequent loading scenarios (Table 3.7). This was not surprising since the loading was increased and the methane yield of dry *Ulva lactuca* is lower than that of the cattle manure. Nevertheless, the methane yield of *Ulva lactuca* in the reactor experiment was higher – when algae biomass made up 20% and 40% of the feedstock – than observed in the batch experiments (methane production from control periods subtracted).

A clear effect on the weight specific methane production was observed when the feedstock was supplemented with *Ulva lactuca*, increasing from 10.6 ml g $feed^{-1}$ (only cattle manure) to 12.8 ml g $feed^{-1}$ from day 48-59 (20% *Ulva lactuca*) and 15.7 ml g $feed^{-1}$ from day 90-105 (40% *Ulva lactuca*). The latter corresponding to a 48% increase when compared to the initial control period. However, increasing the *Ulva lactuca* content in the feedstock to 50% gave no further improvement in the weight specific yield.

The concentration of dissolved sulfate (SO_4^{2-}) of fresh *Ulva lactuca* was 3.9±0,22 g/L. When co-digested with manure the sulfate concentration of the mixture was well below the SO_4^{2-} inhibition level of 1.4g/L, reported by Siles (Siles et al, 2010).

Table 3.7. Operation parameters and performance of thermophilic (53°C) CSTRs treating cattle manure and mixtures of cattle manure and Ulva lactuca. The methane production rate per unit reactor volume was significantly increased when Ulva lactuca was included in the substrate. However, no further increase was observed above 40% algae (dry matter basis). The methane yield of the substrate was lowered when algae were added the substrate.

Feed type	Feed	*Ulva*	OLR	CH_4	CH_4	CH_4 Yield		VFA	pH	COD /N
	TS/VS %	in feed g VS l^{-1}	g VS l^{-1} d^{-1}	Reactor ml l^{-1} d^{-1}	ml g $feed^{-1}$	Total ml g VS^{-1}	*Ulva*	Ac/Pr mM		ratio
Manure	5.3/4.0		2.7	707 ± 20	10.6 ± 0.5	262 ± 20		5/0.5	7.7	37
80% manure: 20% *Ulva*	6.4/4.9	10	3.3	854 ± 69	12.8 ± 0.3	259 ± 8	247 ± 25	7/0.1	7.8	47
60% manure: 40% *Ulva*	8.7/6.6	26	4.4	1049 ± 43	15.7 ± 0.8	238 ± 23	202 ± 20	26/1.6	8.0	48
50% manure: 50% *Ulva*	9.9/7.5	38	5.0	1032 ± 60	15.5 ± 0.9	206 ± 11	150 ± 21	22/5.0	7.8	65

3.3.4 Conclusions on Biogas Part

Ulva lactuca can rather easily be converted to biogas. However, in its raw form the organic methane yield (approximately 180 ml gVS^{-1}) and weight specific methane yield (11-12 ml g^{-1}) is rather modest. Simple maceration can make a significant improvement (> 50%) of the organic methane yield while screw pressing or drying improves the weight specific yield (4-7 times). The results of the reactor experiments clearly illustrated that co-digestion of cattle manure and dry *Ulva lactuca* is possible and that the performance of an anaerobic digester treating cattle manure can be significantly

improved by addition of *Ulva lactuca.* However, an upper methane production limit of approximately 15-16 ml CH_4 g $feed^{-1}$ was also observed, which at the current time seems too little for obtaining an economic feasible production at a Danish centralized biogas plant. However, it should be mentioned that despite the low methane yields of *Ulva lactuca* the total methane potential of *Ulva lactuca* equals or exceeds the potential of many terrestrial energy crops due to a fast growth rate.

3.4 Characterization of Residues and the Potentials as Fertilizers

The objectives of the study were:

1) to determine the fertilizer value of the effluents originating from cattle manure co-digested with *Ulva lactuca* in comparison to the anaerobically digested cattle slurry alone
2) to investigate the potential greenhouse gas emissions (N_2O, CO_2) after application of the different slurries
3) to obtain information about key soil processes underlying the observed effects.

To achieve these aims, a pot experiment with barley plants and a soil incubation study were set up simultaneously. The incubation study was used to intensively follow the evolution of greenhouse gases and key soil processes during the first three weeks after incorporation of the different soil amendments, while the pot experiment was meant to give indication of the longer-term plant response to the effluents. See Chapter 4.2 about basic analysis with regard to fertilizer quality.

3.4.1 Plant Response in the Pot Experiments

The plant dry matter recorded was very similar among all treatments at both harvest dates (21 and 59 days after sowing, Fig. 3.8). Only the treatment receiving mineral fertilizer resulted in a significantly increased dry matter production, whereas all other soil amendments only caused a slight, insignificant increase in dry matter compared to the untreated control.

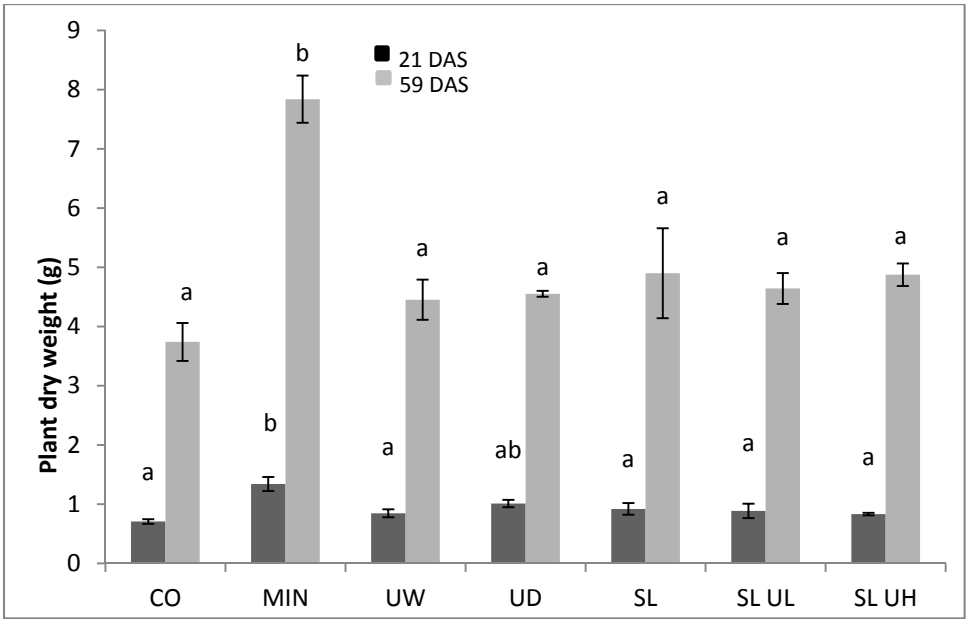

Figure 3.8 Plant dry weight in the pot experiment at two different harvest dates. DAS = days after sowing. CO = non-amended control soil, MIN = soil + mineral fertilizer, UW = soil + wet Ulva lactuca, UD = Soil + dried and ground Ulva lactuca, SL= soil + biogas effluent from cattle manure, SL UL= Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (20%); SL UH = Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (40%). Bars with different letters within the same sampling dates are not significantly different at p≤0,05.

3.4.2 Greenhouse Gas Emissions in the Soil Incubation Study

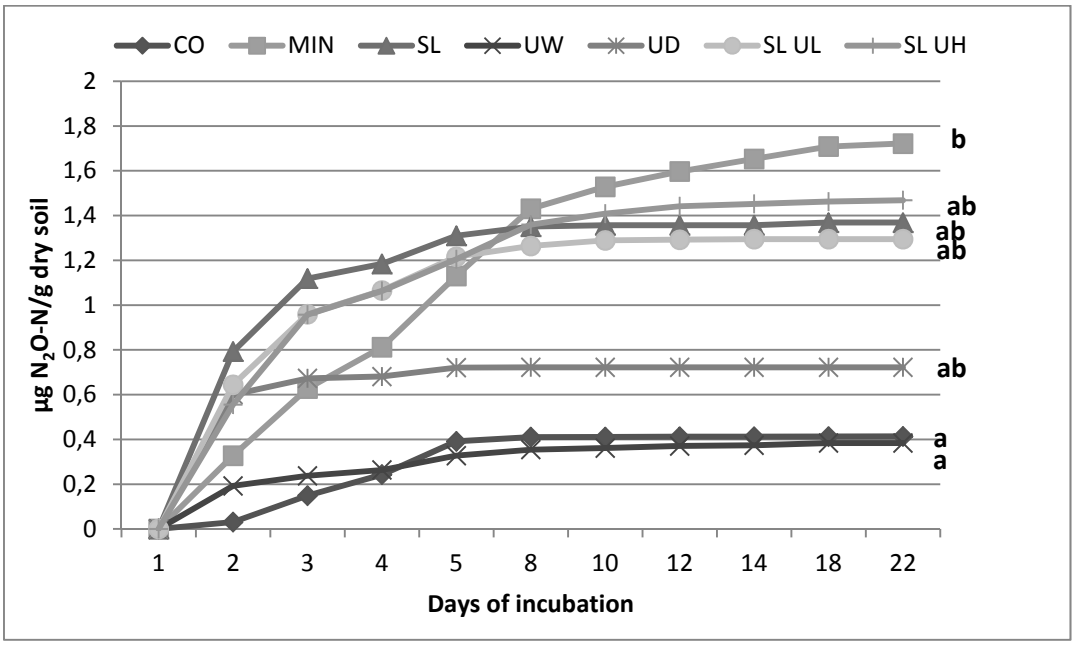

Fig. 3.9 N_2O emissions during soil incubation. CO = non-amended control soil, MIN = soil + mineral fertilizer, UW = soil + wet Ulva lactuca, UD=Soil + dried and ground Ulva lactuca, SL= soil + biogas effluent from cattle manure, SL UL = Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (20%); SL UH = Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (40%).

N_2O emissions were clearly correlated to the mineral N-content of the different soil amendments, being highest in the minerally fertilized treatment and lowest in the non-amended control and pure *Ulva* treatments (Fig 3.9). No differences between the three types of digested slurries were detected. Due to erratic CO_2 measurements at the last sampling dates, CO_2 emissions are shown only for the first two weeks (Fig. 3.10). They did not differ between treatments, except for the treatment that received the dried *Ulva lactuca* powder which caused a significantly higher CO_2 evolution compared to all other treatment except SL. The reason for that can only be speculated about, but might be a high content of easily degradable organic matter in the dried algae. The soils without organic amendment (CO and MIN) had correspondingly the lowest CO_2 emissions. Again, no significant differences between the different slurry types occurred.

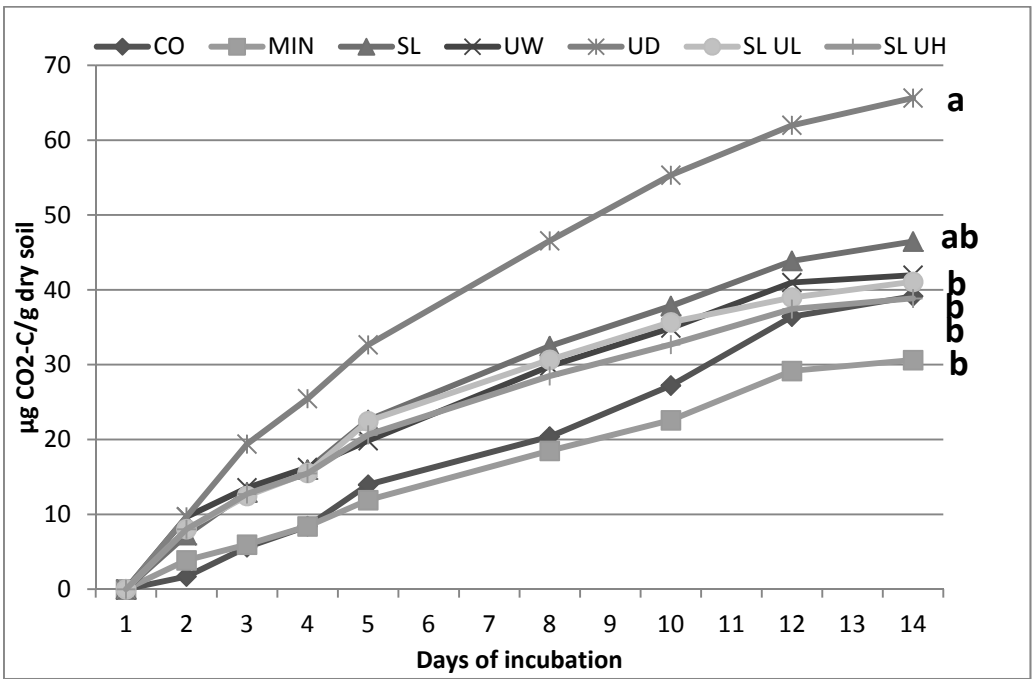

Figure 3.10 CO_2 emissions during soil incubation. CO = non-amended control soil, MIN = soil + mineral fertilizer, UW = soil + wet Ulva lactuca, UD=Soil + dried and ground Ulva lactuca, SL = soil + biogas effluent from cattle manure, SL UL= Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (20%); SL UH = Soil + biogas effluent from co-digested cattle manure with Ulva lactuca (40%).

3.4.3 Preliminary Conclusions

The co-digestion of *Ulva lactuca* together with cattle manure did not alter the overall fertilization value and GHG emission potential of the digestate. However, some deeper insights in plant nutrient uptake and soil nutrient dynamics (including soil microbial biomass) are expected when all data are analysed.

3.5 References

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4 Harvest and Conditioning

4.1 Harvest and Conditioning of Fresh and Dry Ulva lactuca Biomass

4.1.1. Existing Technology

There is extensive experience to harvest aquatic biomass for bioremediation. Among other things, water hyacinths are a big problem in Lake Victoria, where thick mats of water hyacinths cover the lake and causes massive depletion. Also the archipelago of Bohuslän on Sweden's west coast and the Åland islands in the Baltic Sea are particularly plagued by large mats of green algae *Clodophora spp*. and *Enteromorpha spp*. In the Venice Lagoon in Italy large amount of *Ulva Rigida* is collected every year to reduce the negative impact from the macroalgae. Each year 40,000 wet tons are collected in the lagoon. Research has been made to compost the *Ulva* (Cuomo et al, 1994). When these fast-growing plants and macroalgae cover the surface, the bottom vegetation is shaded away because of the massive increase in biomass hypoxia or anoxia occurs, when the biomass decomposes. The result is that the habitats of the fauna change and/or will be destroyed. The harvest takes place from barges/boats that have installed both cut and assembly units (see Fig. 4.1). Some forms of biomass such as water hyacinths and the brown macroalgae *Laminaria* is necessary to cut before they can be removed. When harvest takes place due to bioremediation the macroalgae is normally dumped in landfill and is not used.

Fig. 4.1 Harvest boat at Zürich See (left) and mechanical harvester from CleanLake Inc. in the USA (right) for bioremediation.

Harvesting of macroalgae takes place at industrial scale for production of food additives or hydrocolloids in East Asia, Africa, America and Europe. An example is the production of different species of Carragenan. Production systems for warm water species and cold water species of Carragenan are different; an overwiew is showed in Table 4.1:

Harvesting of macroalgae for food is well-known in East Asia and Japan. China is far the largest producer of macroalgae for food. According to FAO, the world production of macroalgae for all purposes in 2007 was 14.3 million wet tons. The harvest is in general manual with primitive mechanical equipment.

Table 4.1 Overview of production and pretreatment systems for Carragenan species in warm and cold climate (Ref.: Thomas Worm, CPKelco)

Warm water species East Africa South East Asia	1. Producers are fisher families 2. Macroalgae production on nylon lines near the coast 3. Manual harvest 4. Sun dried in the sand or on simple wooden frames 5. Content 60% DM preserved by high NaCl content 6. Pressed in 40 kg bales and packed in sacks 7. Sold to trading company and shipped to Europe 8. Max. storage 2 years before Carragenan deteriorates 9. Washed if the NaCl content is too high 10. Processed in the food processing factory
Cold water species Norway Chile New Foundland Vancouver Island Ireland Bretagne	1. Producers are companies with license up to 20 hectare 2. Wild species are harvested by boat and cut 2-3 meter below surface and collected 3. At low tide special tractors are collecting wild species near the coast 4. The macroalgae is dried in a rotary drum drier 5. Rinsing is needed for stones, shells and crabs 6. At 80% DM pressed in 100 kg bales and shipped 7. Processed in the food processing factory

4.1.2. Manual Harvest for Laboratory Tests

At Seden beach in Odense Fjord large amounts of *Ulva lactuca* are frequently accumulated during the summer period, and therefore it is relatively easy to collect large amounts of biomass. Furthermore, Seden beach is relatively shallow, which makes it possible to collect the sea lettuce without using a boat. The harvest took place from the 2nd to the 4th of June 2009, and 9 persons were involved. The *Ulva lactuca* was primarily lying at the bottom of the sea as a thick carpet of approximately ½-1 m which made it easy to harvest large amounts rapidly. The harvest was carried out with lawn rakes and the *Ulva lactuca* was gathered in vessels before it was placed on europallets to let the excess water drain off before any further transport.

Fig. 4.2 Manual harvest at Odense Fjord

At the harvest, the sea bed was disturbed as little as possible to minimize the contamination from sand and shells etc. Furthermore, other kinds of seaweed than *Ulva lactuca* and other biological material were eliminated. Thus, an almost monoculture of *Ulva lactuca* was gathered. Approximately 1000 wet kilo was gathered in total.

Fig. 4.3 Auger press and Ulva lactuca after pressing but before drying

The *Ulva lactuca* was transported to Danish Technological Institute's Test Facility in Sdr. Stenderup, which has great experience of pretreatment and conditioning of different kinds of biomasses. The *Ulva lactuca* was washed in 7 different containers containing fresh water to eliminate salt (primarily Na, Cl and K) and other foreign particles, e.g. fauna from the surface of the *Ulva lactuca*. Laboratory tests (Bruhn et al. unpub. data) have shown that it is possible to remove all the salt from the macroalgae surface by thorough cleaning in 7 vessels of fresh water, but since the washing comprises considerably larger amounts, it is not expected that all the salt can be removed. Later, ash analyses confirmed that not all the salts were washed out of the *Ulva lactuca*. During the harvest it was not possible to avoid that the *Ulva lactuca* was affected by sand when the particles from the sea bed were stirred up into the water column, and it was impossible to eliminate this contamination completely despite the washing procedure, which is due to the fact that the ash contains a great deal of silicate. Subsequently, the *Ulva lactuca* was pressed mechanically in an auger press making sure that as much water as possible was removed. This process turned out to be extremely suitable for pressing *Ulva lactuca*. The pressing was additionally improved because the *Ulva lactuca* before pressing was pretreated in a grinder that normally is used for grinding of grass. The auger press was adjusted during the process ensuring that the *Ulva lactuca* was pressed the most without choking up the auger. The water content in the *Ulva lactuca* was reduced to 72% from 85%. The water that was pressed out of the *Ulva lactuca* was green; therefore it is assumed that organic compounds were present in the liquid. The final drying took place in drying oven at 105°C. Additional analyses were carried out on the dried *Ulva lactuca*. The dry *Ulva lactuca* was stored in darkness in black bags at room temperature.

4.1.3. Design of Harvesting Equipment

The studies in this chapter show that there are many primitive methods to harvest macroalgae, and all of them are man power intensive. The lack of industrial production systems seems similar to how the fishing sector was developed 100 years ago in Europe

with small boats with or without sail. At that time the equipment was simply nets and lines with hooks. One of the conclusions in this project is that large scale production of macroalgae must take place off shore with modern equipment designed specifically for the purpose and developed from the modern fishing sector. The cost calculation in Chapter 4.4 shows clearly that large scale production systems are needed in order to make macroalgae production economically attractive. Land-based production systems are needed for special purpose as research, breeding of new species and hatcheries.

4.2 Basic Analysis for Combustion Purposes

4.2.1. Discussion of the Basic Analysis

The composition of *Ulva lactuca* varies depending on where it grows and at what point of the season it is harvested. The following analyses do thus only reflect the composition of some representative samples giving an idea on the typical composition of *Ulva lactuca* harvested under Danish conditions. According to the samples investigated the ash content in *Ulva lactuca* can range from 14 % to 50 % depending on where it is grown, the season and if the biomass has been pretreated or not (see Figure 4.4). This is by far higher than typical solid biomass fuels which are used in power and heating plants today and does thus render some challenges (see also discussion Chapter 5.5). In Figure 4.5 the trend clearly reveals that marine biomass in general contains more ash than terrestrial derived biomass. This also includes some common biomass waste from industrial processing such as mask, shea waste, carrageenan waste and olive stones which has been investigated in detail in previous projects (Nikolaisen et al 2005 and 2008)

The highest ash contents in the *Ulva lactuca* samples are found in samples harvested in the sea which are considerably higher than samples harvest from pool trials. This is due to contamination of sand and sea shells clinging to the macroalgae. This explanation becomes obvious when the chemical analyses of the ash composition in Figure 4.6 reveals high amounts of Ca (shell) and Si (sand) compared to the samples from the pool trials. The other cause for the high ash content is high amounts of salts. Some of these salts are remains of the salt water the macroalgae is growing in (high NaCl), but this part can be almost removed by either washing in fresh water or pressing out the water from the algae like in the auger press described in Chapter 4.1.2. In fact the washing in fresh water removes almost all NaCl (see Figure 4.6) from the sample and the ash content of *Ulva lactuca* grown in pool could be reduced from 17 to 14 % and the *Ulva lactuca* grown in sea could be reduced from 50 to 31% (see Figure 4.4).

The high amount of ash as well as the high amount of salt is typical also for other macroalgae than *Ulva lactuca*. In Fig. 4.5 and Fig. 4.7 a comparison between *Sargassum muticium, Chatomorpha linum* and *Gracilaria vermicullophyllum* sampled in Italian water, with *Ulva lactuca* reveals similar composition of the ash mainly as salts although different amounts. It is especially the high amounts of Cl and S that differ these ashes from the terrestrial biomass ashes and industrial biomass waste.

The very high amount of salt in ash could result in problems as it would cause problems in thermal conversion units melting ash (slagging and fouling). However; the high amounts of especially K could also make it valuable as fertilizer.

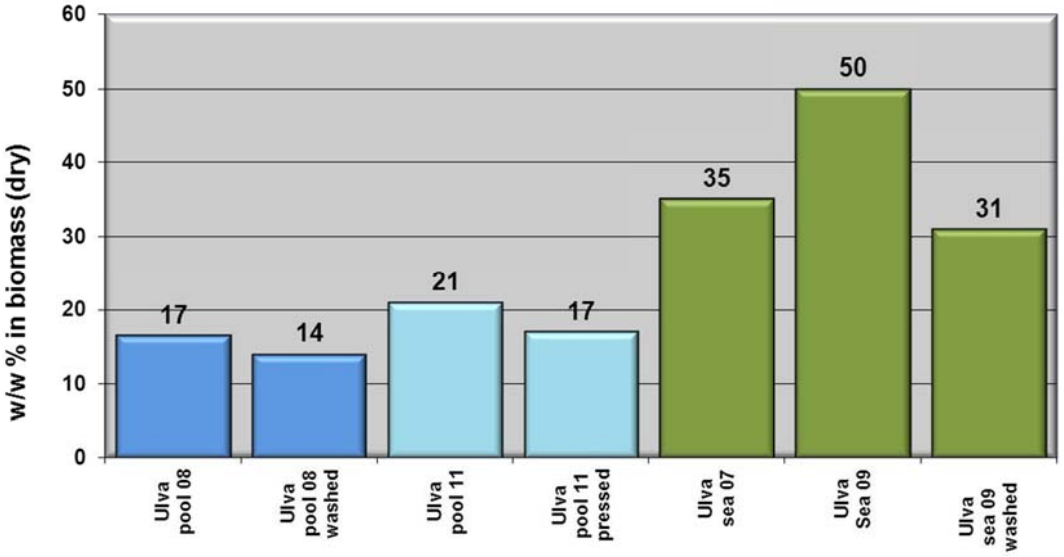

Fig. 4.4 Ash content in Ulva lactuca from different harvest, sources (sea and pool) and treatments (washing with freshwater or pressing in an auger press).

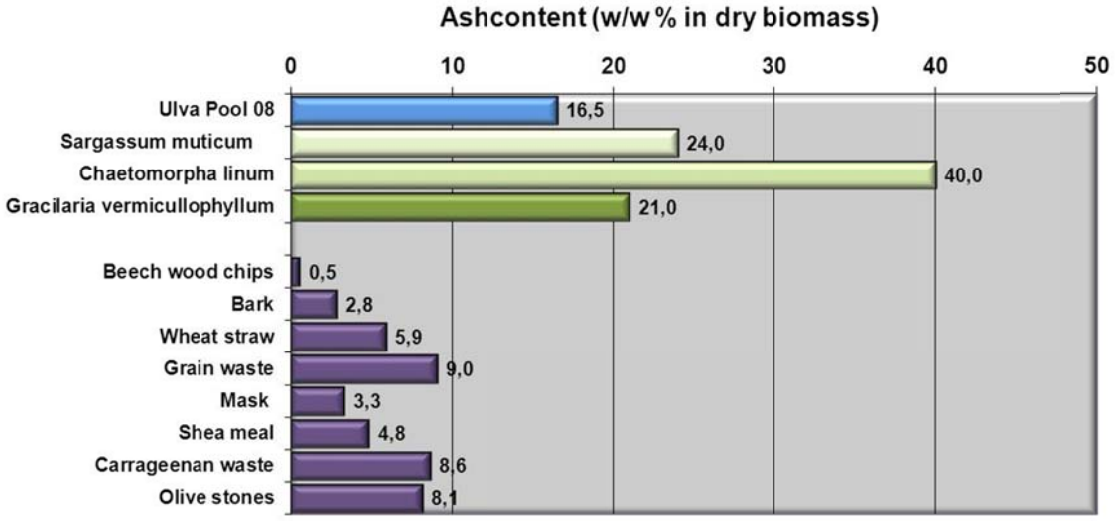

Fig. 4.5 Ash content in Ulva lactuca compared to other macroalgae and solid biomass investigetd in previous projects (Nikolaisen et al. 2005)

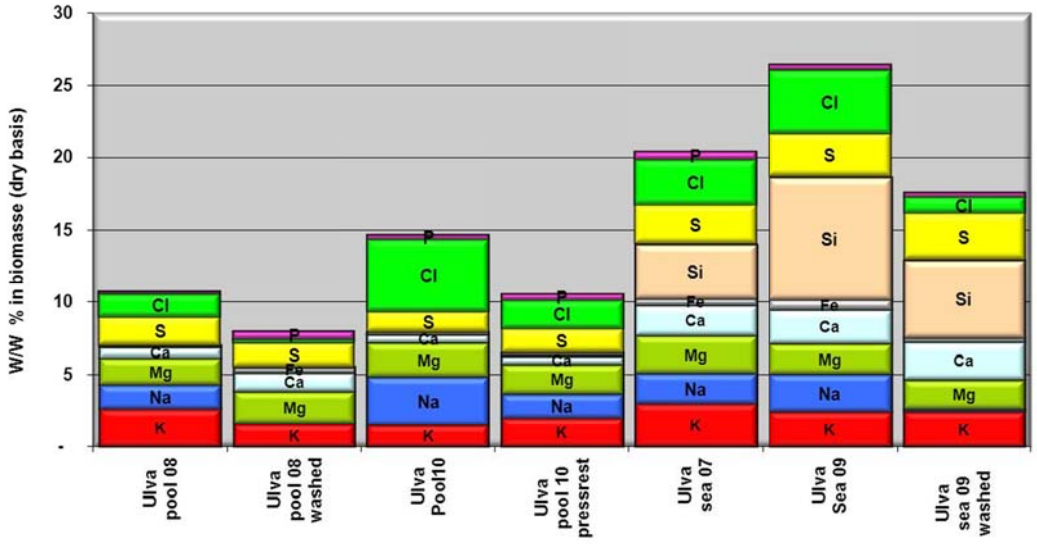

Fig. 4.6 Elements in ash from Ulva lactuca from different harvest, sources (sea and pool) and treatments. Analyses are made by means of WDXRF on ash samples made at 550C. Oxygen is excluded from the ash composition as it is a calculated value giving the rest mass of the ash compared to Fig. 4.4.

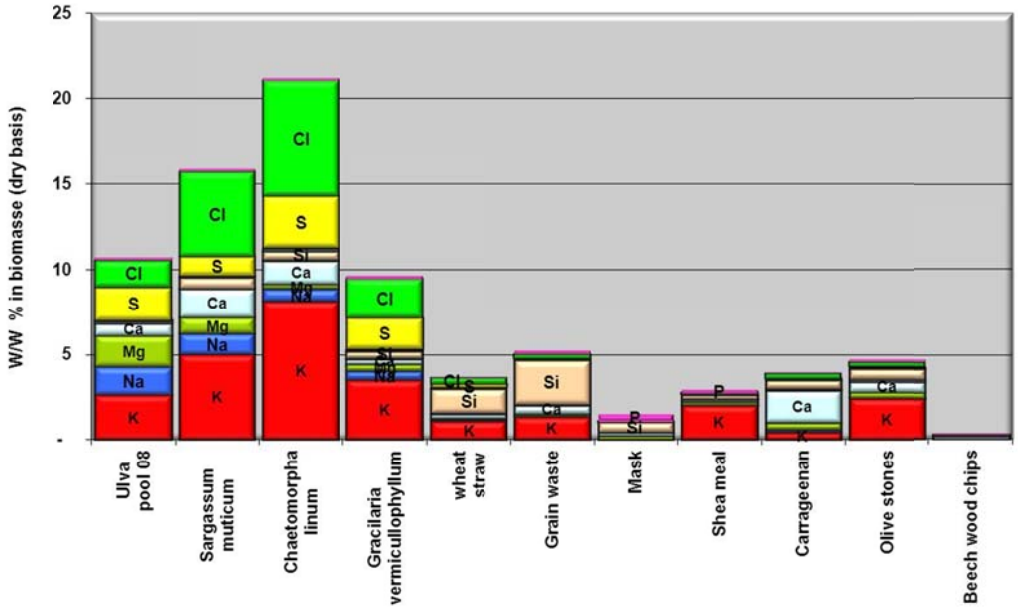

Fig. 4.7 Elements in ash from Ulva lactuca compared to other macroalgae and common solid biomass fuels investigated in previous projects

Even after pretreatment and removal of surface salts, the remaining ash is still a mixtures of salts containing K, S, Ca, Mg and P. These elements are all important nutrients and if extracted or collected after converting the organic part of the algae, these would serve as a high value fertilizer. Analyses of heavy metals revealed the detectable amounts of Zn and Cu, while all others were below the detection limits of the WDXRF (~10 mg/kg). The detection limits of the WDXRF are unfortunately above the limiting values for heavy metals such as Cd (2.5 mg/kg) in bioash according to The

Danish Bioash Order No. 1636. It can thus not be completely ruled out that this limit is exceeded. The same rules do not have limits for Cu and Zn, however there are other rules in force that limit the distribution on residues on farmland which limits Zn with 4000 mg/kg DM and Cu with 1000 mg/kg. The concentrations of these elements were well below the limits in the algae ash from the sea (Cu ~20 mg/kg and Zn ~80 mg/kg).

It can thus be concluded that the ash from algae would be an excellent source for extracting high value fertilizers and should not cause any problems for disposal.

4.2.2 Ash Melting Behaviour

The currently most commonly used method for evaluating the potential melting of ash in thermal conversion units is the CEN/TS 15370-1. The method is a remain of the coal industry based on making an ash of the fuel sample, shaping this ash to a cube or cone that is again gradually heated until the shape of the cube or cones change. From this, three temperatures are defined:

- Deformation Temperature (DT)
- Half sphere Temperature (HT)
- Flow Temperature (FW).

Typically for wood pellets the DT is used and should be above 1200ºC for premium and above 1000ºC for lower qualities.

The DT, HT and FT for the macroalgae samples are listed in Table 4.2. The results reveal mixed results where some of the algae show very low DT, while the *Ulv lactuca a* has temperatures around 1200ºC.

Table 4.2 Ashmelting of macroalgae according to CEN/TS 15370-1

Sample	**DT [°C]**	**HT [°C]**	**FT [°C]**
Ulva lactuca Sea 09	1210	1220	1230
Ulva lactuca Sea 09 Washed	1200	1230	1250
Sargassum muticum	660	700	800
Gracilaria vermicullophyllum	1230	1280	1350
Chaetomorpha linum	540	570	590

The usefulness of the CEN/TS 15370 method is under discussion and can be criticized for not measuring the behavior of the real ash occurring during combustion. Accordingly, Danish Technological Institute has developed a new method, The Slagging Analyzer, which was evaluated and further developed in the PSO project 5297 (Junker et al. 2008) The method is based on a real combustion of the biomass where the behavior of the ash is studied during realistic combustion conditions on a small grate. Test with pellets made from *Ulva lactuca* was carried out but failed due to the fact that the pellets were not possible to ignite in the test unit (see Figure 4.8). This does not necessarily mean that *Ulva lactuca* cannot burn, as similar behavior has been seen with other biomass fuels; e.g. residues from shea nuts do not either burn in the form as pellets, but burn very well when fed as fine powder into the furnace. Combustion of *Ulva lactuca* would thus most likely require the same technology. A pellet with a very

hard surface as *Ulva lactuca* and shea waste is probably the explanation for the bad ignition characteristics.

Fig. 4.8 Combustion and ash melting test of Ulva lactuca pellets in The Slagging Analyser was not successful. The very hard pellet could not ignite and the test was stopped. The same situation is experienced with very hard pellets from shea waste.

Moreover, further analyses of the different macroalgae reveal that they also contain quite high concentrations of N compared to commonly used solid biomass fuels (Fig. 4.9.), which has to be considered as they could cause significant increase in emissions of NOx by combustion and gasification.

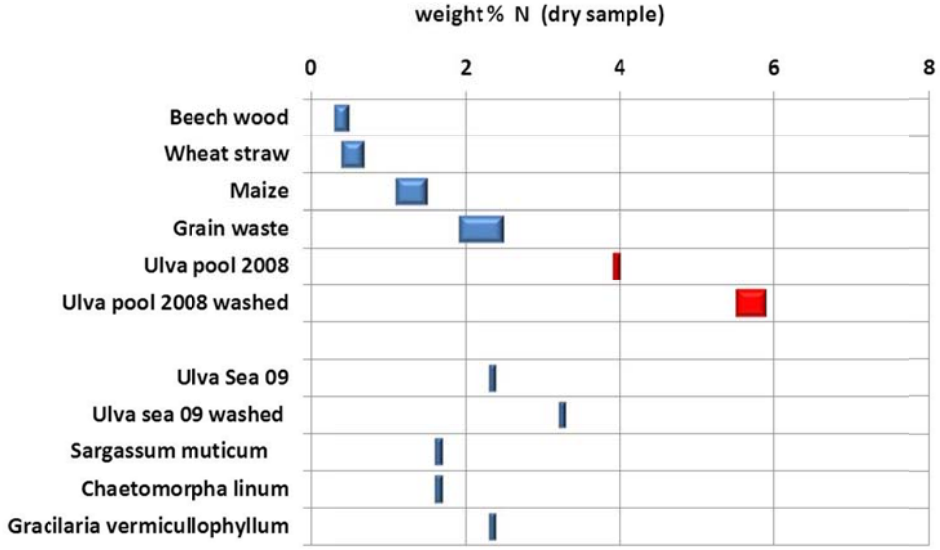

Fig. 4.9 Nitrogen content in macroalgae compared to common solid biofuels

4.3 Conditioning of Ulva lactuca Dry Matter

In order to be able to store or transport macroalgae in an efficient way it has to be dried and densified. Considering this, tests with pre-drying in an auger press, final drying in an oven as well as densifying by pelletization were conducted with *Ulva lactuca*. The drying in the auger press is described in Chapter 4.1.2. In Table 4.3 the mass balance of the wet *Ulva lactuca* processed in the press is revealed. The result shows that the moisture content is only lowered from about 85 % to 72 % by the pressing process. This is far from enough in order to get the *Ulva lactuca* in a storage stable form and further drying is necessary.

However, the pressing does significantly separate 1/3 of the ash with the liquid phase and is thus as rather simple and energy-efficient as a first processing step. The main part of the ash removed is soluble NaCl and some Mg, S, Ca and K as shown in Figure 4.10 This auger press step also reveals to be an important possibility for enhancing the conversion efficiency of the downstream processing of the *Ulva lactuca* although the documentation is not quite clear. (see Chapter 3 concerning biogas and ethanol production).

Table 4.3. Mass balance over the auger press processing of Ulva lactuca. MC is Moisture Content, and d.b. is dry basis.

	***Ulva lactuca* in**	***Ulva lactuca* out**	**Liquid out**
Mass flow	100 kg wet *Ulva*	48 kg pressed *Ulva*	52 kg press liquid
Dry matter	15 kg (85% MC)	13.5 kg (72% MC)	1.5 kg
Ash	3.6 kg (21% d.b.)	2.4 kg (18% d.b.)	1.2 kg

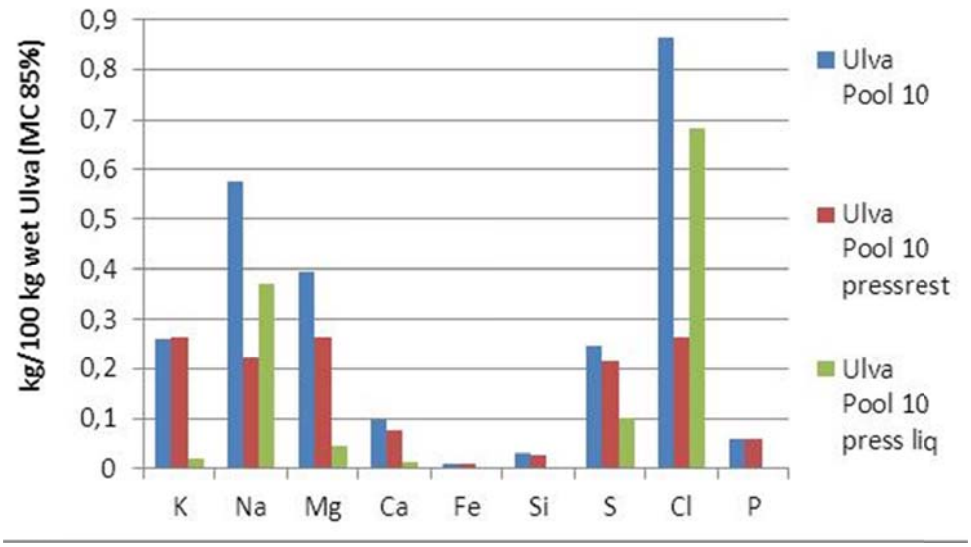

Figure 4.10 Element balance of Ulva lactuca in the auger press

The dried *Ulva lactuca* was in summer 2010 pressed to pellets in an Amandus KAHL pellet press with an ø6 mm flat die at Danish Technological Institute (Fig. 4.11). The dry *Ulva lactuca* is brittle, so milling is not needed. The best result and highest quality of pellets was obtained when the dried *Ulva lactuca* was added water to a total water content of 18-20%. Immediately after the press the water content was approximately

16% and after cooling and initial drying due to rest heat in the pellets from the pelletising process the moisture content was 13-14%.

The pelletising of the *Ulva lactuca* is easy and requires less energy than wood pelletising, but it was not possible to measure an exact energy consumption during the process. No binder was used. The quality of the pellets is high (mechanical stability of 99.5 %) and the bulk density is 760 kg/m3 (ø6 mm pellets). Calculated with a moisture content of 16.5% and an ash content of 28.5 %, the energy density (NCV) would be about ~7900 MJ/m^3 (or 10.4 MJ/kg) which can be compared to 12700 MJ/m^3 wood pellets (650 kg/m^3, 0.5 % ash, 10% MC).

Fig. 4.11 Production of Ulva lactuca pellets on Amandus KAHL pellet press at Danish Technological Institute.

4.4 Cost Calculations of Dry and Wet Ulva lactuca as a Biomass Ressource

4.4.1 Cost Calculation without CO_2 Injection

The systems for production of *Ulva lactuca* in basins at the size of 1 hectare at a power plant is described in Chapter 5.2, 5.3 and 5.4. In this Chapter an evaluation of the economy related to growth, harvest and conditioning processes is conducted. The cost for dry *Ulva lactuca* as solid biofuel is calculated and compared to other solid biofuels. The costs for wet *Ulva lactuca* for methane production are calculated and the income for heat and electricity production is compared with the expenditures. The calculations include the following traditional steps:

- Estimation of the capital costs for the basins, buildings and machinery
- Estimation of the operational costs for dry and wet *Ulva lactuca* production
- Estimation of the total cost without and with CO_2 injection
- Comparison of the prices with similar products for energy production.

Capital costs and operational costs have been estimated on the basis of budgets from industrial suppliers of basins, aquasystems for fish farming, conveyor belts, and driers. Energy supply for the drying process is low pressure steam from the power plant. The cost calculations are show in Tables 4.4, 4.5, 4.6 and in Annex 2.

Table 4.4 Prerequisites for cost calculation of wet and dry Ulva lactuca.

Prerequisites for cost calculations for 1 hectare of basins without CO_2 injection	
Depreciation and interest	15 years, 4 %
Annual operation for basins	7000 hours
Harvest hours	Once a week for 8 hours. Total 400 hours/year
Maintenance cost	2 % of capital cost annually
Cost of electricity	0.40 DKK/KWh
Ulva lactuca wet production	400 tons/ ha annually
Ulva lactuca dry production	50 tons DM/ha annually
Ulva lactuca dry value	500 DKK/ton DM
Ulva lactuca metane production	4000 m^3 for CHP production
Wages dry *Ulva lactuca*	1½ man year: 600,000 DKK
Wages wet *Ulva lactuca*	1 man year: 400,000 DKK
Electr. for water, pumps paddles	62 kW installed. Used 7000 hours/year
Electr. for harvest and conveyors	25 kW installed .Used 400 hours/year
Electr. for drying line	40 kW installed. Used 400 hours/year
Steam for drying	251 MWh to dry 400 wet tons to 10% humidity

Table 4.5 Investment for 1 hectare production facilities for Ulva lactuca

Investment in 1000 DKK	**Dry *Ulva***	**Wet *Ulva***
4 raceways each 2500 m^2	9300	9300
Water supply, pumps, paddles	1200	1200
Harvest system and conveyors	1500	1500
Storage tank for fertilizer	100	100
Drying line	2000	0
Building for drying line	450	0
Building for storage of dry *Ulva lactuca*	450	0
Total investment in 1000 DKK	**14,100**	**12,100**

Table 4.6 Annual cost for 1 hectare production facilities for Ulva lactuca without CO_2 injection compared to the value of the Ulva production for energy purpose

Annual costs in 1000 DKK	**Cost type**	**Dry *Ulva***	**Wet *Ulva***
Depriciation	Capital	1000	807
Average interest	Capital	300	242
Wages	Operational	600	400
Maintenance	Operational	300	242
Electricity	Operational	184	178
Water, chemicals, fertilizer	Operational	100	100
Steam for power plant for drying	Operational	33	0
Annual costs in 1000 DKK		**2517**	**1969**
Annual value of *Ulva lactuca* for energy		**25**	**20**

It is clear from these calculations that a concept where the only outcome of the system is biomass for energy purposes is far too expensive compared to the value of the biomass produced. The annual costs for the 1 hectare system amount to 2,517,000 DKK and the income by selling the *Ulva lactuca* as fuel for a power plant is 25,000 DKK which is the price for the same amount of straw delivered at the power plant. Producing wet *Ulva lactuca* for a biogas CHP plant can produce 4000 m^3 of methane annually, and this amount of gas can give an income by producing heat and electricity of 20,000 DKK. The annual expenditures are 1,969,000 DKK. The conclusion is that there must be extraction of high value products from the macroalgae before end use for energy and the calculated system is far too small; thus there must be designed much larger production systems.

4.4.2. Cost Calculation for CO_2 Injection

The injection of CO_2 is described in Chapter 5.2 and the cost calculation is made as an **additional** price to the price calculated in Table 4.6. The injection system is made as a scrubber system. Capital and operational costs for the scrubber system have been roughly estimated for algae basin areas of 1 hectare and 100 hectares. Main results are shown in Table 4.7.

Table 4.7 Cost calculations for CO_2 scrubber system. Note that the additional algae production is optimistic with an additional production of 50 tons DM/year by CO_2 addition.

Prerequisites for cost calculations for 1 hectare and 100 hectares				
Depreciation		15 years, 4 %		
Annual operation		3500 hours (no operation during the night)		
Cost of electricity		0.40 DKK/kWh		
Maintenance cost		2 % of capital cost annually		
Distance from algae bassins		500 meter		
Additional algae production		50 tons DM/ha annually		
Algae value		500 DKK/ton DM		
Operational data		**1 ha**	**100 ha**	
Salt water flow	m^3/h	500	50,000	
Flue gas flow	Nm^3/h	5,000	500,000	
Power consumption	kW	101	3,711	
Additional algae production	Tons DM/year	50	5,000	
Annual costs in 1000 DKK		**1 ha**	**100 ha**	
Depreciation and interest	Capital	730	13,900	
Electricity	Operational	140	5,200	
Wages	Operational	180	180	
Maintenance	Operational	160	3,090	
Total costs		**1200**	**22,370**	
Value of additional algae production		**25**	**2,500**	

The total annual costs for the 1 hectare system inclusive CO_2 injection are 3,717,000 DKK and the income by selling the *Ulva lactuca* as fuel for a power plant is 50,000 DKK which is the price for a similar amount of straw delivered at a power plant. Wet *Ulva lactuca* for a biogas CHP plant can produce 8000 m^3 of methane annually, and this amount of gas can give an income by producing heat and electricity of 40,000 DKK. The annual expenditures are 3,168,000 DKK.

It is clear from these estimates that the value of the additional algae production obtained from transfer of CO_2 from flue gas is very much lower than the capital and operational costs of the scrubber plant. It can be concluded that although it is technically possible to increase the algae production by a flue gas scrubber system, this process is not economically feasible.

The costs can be reduced considerably by distribution of flue gas directly to the basins. A rough calculation shows that the total annual costs can be cut down to only 20% of the scrubber system. However, this is still too high costs in comparison with the added value of algae production and as mentioned in Chapter 5.2 this solution is not considered to be environmentally acceptable.

The total price for dry *Ulva lactuca* for combustion or gasification is around **3,100 DKK/GJ** compared to straw: **35 DKK/GJ** and wood pellets: **65 DKK/GJ**. The conclusion is that this concept is not economically realistic when *Ulva lactuca* is only grown for energy purposes. The same conclusion is valid for wet *Ulva lactuca* for methane and bioethanol production.

4.5 References

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Nikolaisen, L., Jensen, T.N., Hjuler, K., Busk, J., Junker, H., Sander, B., Baxter, L., Bloch, L.. 2002: Quality Characteristics of Biofuel Pellets. PSO project no. 1996.

Nikolaisen, L., Busk, J., Hjuler, K., Jensen, P, A., Jensen,T, K., Bloch, L.:2005: CO2 neutral Fuels for Power Plants. PSO project no. 5075

Nikolaisen, L., Hinge, J., Christensen, I., Jensen, P, A., Dahl, J., Birkmose, T, S., Sander, B., Kristensen, O: 2008: Utilization of Ash Fractions from Alternative Biofuels used in Power Plants. PSO project no. 6356

5 Integration of Algae Production in Power Plants

5.1 Flue Gas Quality for Algae Growth

In order to obtain high dry matter production capacities in algae production basins, the limitation of CO_2 transport from the atmosphere to the algae basins can be eliminated by supply of flue gas from power plants. Flue gases contain high amounts of CO_2 from combustion of fuels like coal, oil, gas, wood and straw. Another potential concentrated CO_2 source is off-gas from ethanol production plants.

Flue gas from three types of combustion plant gas is considered in this project:

- Coal-fired power plant equipped with deNOx-plant, ash removal system and desulphurisation plant (pulverised fuel)
- Wood chips-fired combined heat and power plant (grate firing)
- Straw-fired combined heat and power plant (grate firing).

Typical compositions of flue gases and bioethanol off-gas are shown in the table 5.1.

Table 5.1. Typical flue gas compositions. All values on dry basis. NO_x is primarily present as NO, which has low water solubility.

Parameter	Unit	Coal	Wood	Straw	Off-gas
N_2	vol-%	80	78	79	2,5
CO_2	vol-%	13	15	14	96
O_2	vol-%	6	6	6	<0,6
Ethanol	vol-%				0,6
SO_2	mg/Nm^3	30	10	100	
HCl	mg/Nm^3	1	2	50	
NO_x	mg/Nm^3	100	200	300	
Particulates	mg/Nm^3	10	10	10	
Hg	µg/Nm^3	3	0,05	0,3	

In the project application it was proposed to distribute flue gas in the algae basins. However it has been realized that emission of flue gases from basins close to the ground is environmentally unsafe and will probably not be accepted by the authorities. Collection of gases from the basins is not technical/economically feasible. As an alternative it is proposed to transport salt water from the basins to a flue gas scrubber placed at the power plant. Such a system is described in the next section. This also eliminates problems with condensation and corrosion in a flue gas channel delivering flue gas to the basins.

5.2 Scrubber System for CO_2 Transfer from Flue Gas

As mentioned in Chapter 5.1 the preferred method for transfer of CO_2 from flue gas to algae growth is transportation of salt water from the basins to a flue gas scrubber. This system comprises the following major components:

- Piping for transportation of salt water from basin to scrubber and back

- Salt water pump from algae basins to scrubber
- Salt water pump from scrubber to algae basins
- CO_2 flue gas scrubber
- Booster fan for flue gas from the Flue Gas Desulphurisation (FGD) plant
- Flue gas channel from FGD plant to CO_2 scrubber and from scrubber to stack.

For technical design of this plant two basic pieces of information are required:

- Flow rate of salt water to scrubber
- Flow rate of flue gas to scrubber.

The flow of salt water depends on the amount of CO_2 to be transferred. It is assumed that on the basis of an annual algae dry matter production of 100 tons/ha the annual consumption of CO_2 is 200 tons/ha or in mean 25 kg CO_2/ha/hour. However, as the growth rate vary a lot during the day and the season the scrubber system is designed for 100 kg CO_2/ha/hour.

The flow of salt water required for transfer of this amount of CO_2 depends on the solubility of CO_2 in salt water. Based on (Weiss R.F. 1974) the solubility of CO_2 in salt water with a salinity of 3 % is 0.034 moles/l/atm at 20°C. At a CO_2 partial pressure of 0.13 bar in flue gas (Table 5.1) the CO_2 solubility is 0.0044 moles/l corresponding to 0.2 gram CO_2/l. Combined with the required maximum CO_2 transfer of 100 kg CO_2/ha/hour the calculated maximum flow of salt water is 500 m^3/hour/ha.

For typical liquid/gas (L/G) ratios in scrubbers there is a high surplus of CO_2 in the flue gas compared to the solubility of CO_2 in salt water. This means that high L/G ratios can be applied and in this case the scrubber design is based on a L/G value of 100 l/Nm^3, corresponding to a flue gas flow rate of 5000 Nm^3/hour/ha. This L/G ratio is too high for a conventional spray scrubber design and submergent scrubber technology, where flue gas is bubbled through the liquid, is preferred.

A flow diagram of the CO_2 transfer plant is shown in figure 5.1.

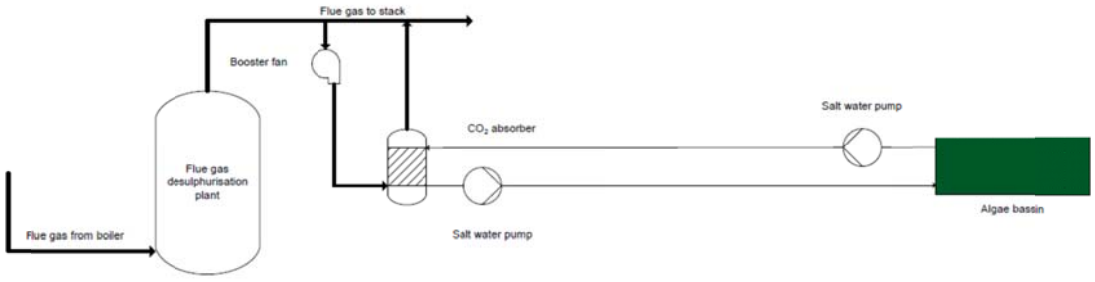

Fig.5.1. CO_2 transfer plant

5.3 Heat Supply from Power Plant to Algae Basins

During winter time the low temperature limits the algae production rate. This may be overcome by supplying heat from the power plant to increase the temperature in the algae basins.

The amount of energy required to heat up the algae basins is however high. As an example a basin with an area of 1 ha and a depth of 0.3 m contain 3,000 tons of water. To increase the temperature in the basin with 5°C one time requires an amount of energy of approximately 70 GJ/ha. This has to be done many times during the winter. In comparison an additional algae production of 10 tons dry matter/ha has a lower heating value on dry basis of 140 GJ/ha. From both an energy and an economical point of view it is unfeasible to apply heating of algae basins for increased production rates.

5.4 Design of Basins (raceways) for Algae Production in Power Plants

Basins (or raceways) in the total size of 1 hectare are designed to give an idea of the equipment needed and the costs for investment and the running costs. 4 basins are designed, each in a size of 2500 m^2 with a length of 100 meter and a width of 25 meter. The basins are made of concrete with a height of 0.6 meter, and the depth of the water is 0.3 meter. The bottom of the basins is flat. The basins are arranged two and two besides each other with a distance of 15 meter to make piping and transport of macroalgae simple (see Annex 8). The piping for salt water is arranged in the middle between the 4 basins. The distance to the salt water intake is up to 400 meter and the height above sea level is maximum 5 meter.

The rentability of macroalgae cultivation in raceway ponds relies on maximization of the biomass production with a minimal input of energy and manpower. Thus, automatic control of a number of parameters, such as water flow and addition of nutrient and CO_2, is crucial. A paddle system in each raceway pond secures a water circulation of approximately 20 cm s-1. In order to save energy, circulation velocity may be lowered at night. Nutrients will be supplied from one central 200 m^3 manure tank. In each raceway,5 automatic feed pumps will control the addition of manure to a concentration of approximately 20 µM NH_4+-N and 5µM P. Addition of nutrients will take place in one pulse over night to minimize the competition for nutrients by microalgae present in the ponds. In order to optimize the growth of the macroalgae, CO_2 will be added in the form of flue gas. The flue gas will be added through a scrubber system, where a pH controlled automatic valve will adjust the CO_2 addition keeping the pH of the water in the range between 6.5 and 8.5. A freshwater as well as a seawater intake will supply new water, in order to make up for the water that leaves the system through evaporation and harvest. A level sensor in each pond will control the water intake, and the inflowing water will pass through a drum filter before reaching the pond. Depending on the water temperature and the amount of available light, the algae production and the harvestable biomass will fluctuate over the year. The standing stock of the biomass will be continuously monitored via light sensors in the tanks. Based on calculations of the seasonal optimal biomass density in the tanks, the harvestable biomass per week will be estimated in order to maximize the production.

Fig. 5.2 Example of harvesting with conveyor band

The harvest equipment which is a conveyor band is placed at each basin and is submerged during harvest. Fig. 5.2. The speed of the water transports the macroalgae to the harvester. Harvest takes place once a week and the total amount to be harvested is around 400 wet tons annually for 1 hectare basins. The amount per harvest is 8 tons. The macroalgae is transported by conveyor band to a drum drier. Dewatering takes place on the band and the water content is maximum 80% entering the drum drier. To make the dewatering process on the band stainless steel should be applied. The operating time for the drier is minimum 8 hour. Start-up takes 1 hour, shut down time is ½-1 hour. The capacity of the drier must be up to 1000 wet kg/hour drying from 80% to 10% water. The capacity of the drier is 7-800 kg evaporation/hour which is a fairly small drier compared to commercial driers in the fodder business where the capacity is 15-20,000 kg evaporation/hour.

5.5 Use of Dried Algae in Power Plants

5.5.1: Co-firing of Dried Macroalgae in Power Plants

Co-firing of biomass in coal-fired power plants is a proven technology for CO_2-reduction. The co-firing potential depends on the physical and chemical properties of the biomass product, i.e. moisture content, particle size and content of ash, alkali, chloride and other components. From a combustion point of view dried macroalgae powder is suitable for pulverized fuel co-firing, but as shown in Chapter 4.2 the content of ash, alkali, chloride and sulphur is very high. Typical fuel properties of dried *Ulva lactuca* powder, straw and coal are shown in Table 5.2.

Table 5.2 Typical fuel properties of Ulva lactuca and coal

Parameter	**Unit**	***Ulva lactuca***	**Straw**	**Coal**
Moisture	%	14	14	14
Lower heating value, as received	kJ/kg	11.4	15	24
Ash	% dry basis	16.5	4.5	12
Si	% dry basis	0.02	0.8	3
Al	% dry basis	0.0	0.005	1.5
Fe	% dry basis	0.13	0.01	0.6
Ca	% dry basis	0.7	0.4	0.3
Mg	% dry basis	1.8	0.07	0.15
K	% dry basis	2.6	1.0	0.2
Na	% dry basis	1.6	0.05	0.05
S	% dry basis	2.0	0.12	0.7
Cl	% dry basis	1.6	0.4	0.05
P	% dry basis	0.16	0.06	0.02
Br	% dry basis	0.03		

On a heating value basis the content of Ca, Mg, K, Na, S and Cl in *Ulva lactuca* is very high in comparison with coal and also much higher than in straw. By co-firing of *Ulva lactuca* in coal-fired power plants the content of Mg, K and Na in the fly ash and the content of SO_2 and HCl in the raw flue gas will be significantly increased. The share of *Ulva lactuca* co-firing is limited by the impact on slagging, catalyst deactivation, corrosion, emissions and residue quality (fly ash, bottom ash, gypsum). It is expected that the influence on the fly ash quality is the most critical factor and a calculation for 0-20 % co-firing on mass basis has been performed. In Table 5.3 the results are compared with the critical quality requirements for fly ash used in concrete according to the European standard EN450-1.

Table 5.3 Critical fly ash quality parameters by Ulva lactuca co-firing. Ulva lactuca-% on mass basis

	0 % Ulva	**5 % Ulva**	**10 % Ulva**	**15 % Ulva**	**20 % Ulva**	**EN 450-1**
Alkali (Na_2O+0.658*K_2O)	2.0	3.8	5.7	7.5	9.3	<5
MgO	2.2	3.4	4.6	5.9	7.2	<4

The influence on the content of alkali and MgO is substantial and the ash quality standards are exceeded even by 10 % *Ulva lactuca* on mass basis, corresponding to 5 % on energy basis. In comparison, by co-firing of 20% straw on mass basis the content of alkali is increased to only 3.6% and there is no significant change in the content of MgO. It is concluded that the use of *Ulva lactuca* powder as direct co-firing fuel in coal-fired power plants is very limited.

5.6.2: Gasification of Dried Macroalgae in Power Plants

The limitations mentioned in Chapter 5.6.1 may however be overcome by new technology. Low-temperature circulating fluidized bed gasification (LT-CFB) for biomass with a high content of ash, alkali and chloride is to be demonstrated in 6 MW-

scale. With this technology + 90 % of the ash is separated from the gasified fuel ahead of co-firing and allows high shares of high-alkali biomass to be co-fired (see Annex 6).

5.6.3: Combustion of Dried Macroalgae in Special Designed Power Plants
Low quality biomass is burned on step grates where the fuel is pushed through the combustion chamber by moving grate bars. Low quality biomass is household waste, bark or wood chips with high moisture content up to 55-60% water and in addition often a high ash content. Fig. 5.3. The macroalgae can be burned on this type of grate after pretreatment where the water content is reduced from 85% to 60%. An auger press can reduce the moisture content to around 70% and drying on a band drier can reduce the moisture content to 60%. The heating value of the biomass is low, below 9 MJ/kg, and it is necessary to have a thick fuel bed on the grate, up to 40-50 cm, in order to feed enough energy into the grate. The thick fuel bed is overturned on the inclined grate by gravity and by the moving grate bars. This is to secure that all biomass is burned out at the end of the grate. The combustion chamber is lined with refractory which keep a high temperature during the combustion process. The large amount of water in the biomass is evaporated, mainly by radiation from the very hot refractory, but also by radiation from the burning biomass. This type of grate is built in sizes from 1 MW to 100 MW where 1 MW is the size of farm scale boilers and 100 MW is the size of combined heat and power plants.

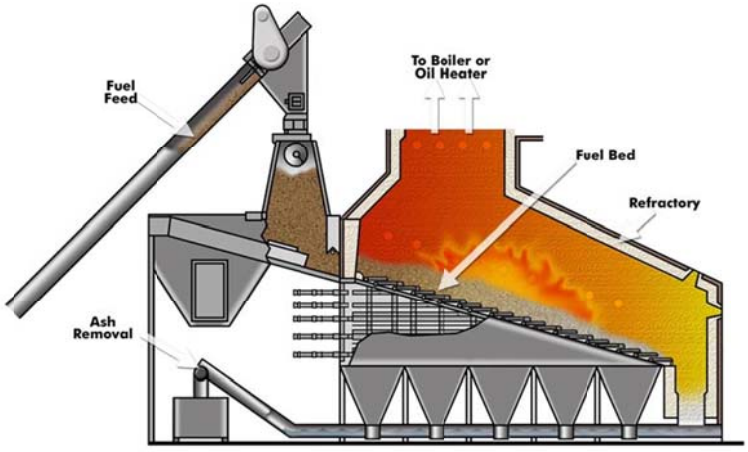

Fig. 5.3 Typical step-grate designed for low quality biomass with high moisture and/or ash content. Low quality biomass is household waste, bark or wet wood chips. The moisture content is maximum 55-60%. This type of grate could be used to burn macroalgae.

5.6 References

Weiss R.F. 1974. Carbon dioxide in water and seawater: The solubility of non-ideal gas, Marine Chemistry, 2 (1974) 203-15.